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Abstract	<p>Remediation using nanoparticles depends on proper documentation of safety aspects, one of which is their ecotoxicology. Ecotoxicology of nanoparticles has some special features; while traditional ecotoxicology aims at measuring possible negative effects of more or less soluble chemicals or dissolved elements, nanoecotoxicology aims at measuring the toxicity of particles, and its main focus is on effects that are unique to nano-sized particles, as compared to larger particles or solutes. One of the main challenges when testing the ecotoxicity of nanoparticles lies in maintaining stable and reproducible exposure conditions, and adapting these to selected test organisms and endpoints. Another challenge is to use test media that are relevant to the matrices to be treated. Testing of nanoparticles used for remediation, particularly redox-active Fe-based nanoparticles, should also make sure to exclude confounding effects of</p>

altered redox potential that are not nanoparticle-specific. Yet another unique aspect of nanoparticles used for remediation is considerations of ageing of nanoparticles in soil or water, leading to reduced toxicity over field-relevant time scales. This review discusses these and other aspects of how to design and interpret appropriate tests and use these in hazard descriptions for subsequent risk assessments.

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Keywords

(separated by '-')

Environment - Nanoparticles - Organic pollutants - Polluted soil - Toxicity

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# Chapter 28 1

## Ecotoxicity of Nanomaterials Used 2

### for Remediation 3

Claire Coutris, Alena Ševců, and Erik J. Joner 4

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## 24 28.1 Introduction

25 Ecotoxicology for evaluating possible negative effects of nanomaterials  
26 (nanoecotoxicology) has some special features; while traditional ecotoxicology  
27 aims at measuring possible negative effects of more or less soluble chemicals or  
28 dissolved elements, nanoecotoxicology aims at measuring the toxicity of particles. In  
29 addition, nanoecotoxicology has its main focus on those effects that are unique to  
30 nano-sized particles, as compared to larger particles or solutes. For this reason,  
31 experiments in nanoecotoxicology usually compare the results with effects caused  
32 by larger particles with similar composition. Another common comparison is that to  
33 the effects of dissolved ions of the same elements constituting the nanoparticles  
34 tested, since many metal-based nanoparticles may partly dissolve, and the toxic  
35 effects can be due to their soluble ionic component. These so-called control treat-  
36 ments are not always easy to establish, or they may result in imperfect comparisons,  
37 as larger-scale particles (often referred to as “bulk material”) may behave quite  
38 differently due to their larger size, and soluble salts of elements found in many  
39 nanomaterials may not exist, or may precipitate during the tests (Kahru and  
40 Dubourguiet 2010; Handy et al. 2012a; Sørensen et al. 2015).

41 Exposing organisms to nanomaterials requires stable suspensions of these  
42 nanomaterials. This is typically obtained through the use of surface-active agents  
43 reducing the attractive forces between particles (Labille and Brant 2010), causing  
44 them to remain suspended in water or other media for a period of time that would  
45 permit absorption or other interactions causing harm to the test organism. These  
46 surfactants may themselves affect the test organisms, and thereby the test outcome.  
47 Control treatments used for comparisons should therefore take this into account.

## 48 28.2 Toxicity of Particles

49 Particle toxicity can be rather different from toxicity of soluble substances or ions.  
50 This is due to the strong barriers against the uptake of particles that most organisms  
51 possess. Ions enter organisms through channels (transporters) in the cell mem-  
52 branes, which can discriminate the uptake based on characteristics like charge  
53 and size. Organic molecules may either pass through uptake channels for organic  
54 nutrients or cross bilayer membranes due to their hydrophobicity. Nanoparticles fit  
55 none of these routes of transport, and are therefore relegated to entering cells by  
56 random “back doors”, like compromised cell membranes or accidental passive  
57 uptake. The size of the particles in question is then of course of major importance  
58 for such uptake. Most nanoparticles used for remediation are found among the  
59 larger nanoparticles, typically >50–200 nm. To put this in perspective, a 20 nm  
60 silver nanoparticle would contain 750,000 atoms (Oughton et al. 2008), each atom  
61 approximately twice as big as, e.g., a zinc ion that enters cells through ion channels.

Needless to say, most of the regular paths for entering into cells are not permitting the entry of nanoparticles. One exception here may be endocytosis.

Yet, some nanoparticles find their way through cell walls and membranes, and end up inside cells. Here they represent quite a different type of toxicity than dissolved ions of the same elements, partly because they constitute discrete particles rather than diffusive ions that may spread among cells. A particle is likely to stay inside the cell it has entered and end up in lysosomes (in the case of eukaryotic cells), but may, e.g., dissolve and represent a steady source of dissolved ions that may be harmful. The concentration of such ions is likely to be substantially higher in a cell containing a nanoparticle than in a cell being exposed (along with neighboring cells) to a similar level of dissolved elements that may move more or less freely within the exposed tissue.

Nanoparticle toxicity mechanisms include effects of dissolved ions from metallic nanoparticles (common for Ag nanoparticles that release  $\text{Ag}^+$ ), induction of reactive oxygen species (ROS and other types of oxidative stress; such effects have been shown for iron-based nanoparticles (Lewinski et al. 2008)) and damages directly related to the surface and shape of nanoparticles (e.g., nanotubes and their asbestos-like induction of damages on cells). For Fe-based nanoparticles, only mechanisms related to ROS-formation and oxidative stress have been described as direct effects. These have been reviewed recently (Lei et al. 2018), and will not be detailed further here. Indirect effects on  $\text{O}_2$  availability is another mechanism, but as we argue below, this is not a nanoparticle-specific toxic effect.

### 28.2.1 Ageing and Other Time-Dependent Modifications of Toxicity

A particular aspect of nanoparticle toxicity testing that is relevant for nano-sized zero-valent iron (nZVI) and other nanoparticles to be used for remediation is reduction in toxicity over time. While all nanoparticles are subject to changes in surface properties as a result of interactions with environmental matrices, nanoparticles for remediation are frequently designed to lose their reactivity by interacting with environmental pollutants. Including temporal changes in toxicity during testing is therefore a particularly relevant aspect that should be assessed for such materials. Reduced toxicity as a result of ageing in soil has indeed been demonstrated for nZVI aged for 30 days in soil, using growth (body weight) of earthworms as an endpoint (El-Temsah and Joner 2012a) and partly for rice after 2–4 weeks ageing (Wang et al. 2016). In the natural environment, living organisms will mostly be exposed to aged nZVI, and not to pristine particles. This is important to keep in mind when designing toxicity studies. Not only may such tests show that adverse effects of the nanoparticles are short-lived, but it may also be helpful in designing nanoparticles for remediation as toxicity and reactivity against pollutants are likely to be strongly linked (Hjorth et al. 2017).

102 Different types of nanomaterials may affect organisms differently. This is yet  
103 another aspect to consider when choosing how a given nanomaterial is tested with  
104 respect to ecotoxicity. For approval of new nanomaterials or for conducting risk  
105 assessments, a set of minimum three tests with contrasting organisms must be carried  
106 out (Baun et al. 2009).

### 107 **28.3 Choice of Test Organisms**

108 The choice of test organisms is important for several reasons, and may ultimately  
109 determine the outcome of a testing scheme. First, the choice of organisms must be  
110 relevant for the matrix to be treated. If nanomaterials for treating polluted soil are to  
111 be tested, soil organisms should be chosen. Similarly, freshwater and marine organ-  
112 isms are relevant to their native habitats. Within these three major organism habitats,  
113 there may be some overlap, or it may be relevant to include organisms from two  
114 groups as a remediation situation can affect more than one matrix: treated soil may  
115 lead to nanomaterials ending up in nearby ponds and streams, or streams and rivers  
116 may reach brackish or saltwater habitats.

117 When choosing test organisms within these major groups, there are at least three  
118 key aspects to consider:

- 119 • How contact with the tested material may occur
- 120 • Which endpoints are available to assess effects
- 121 • Which trophic level the organism belongs to (and how this will affect exposure).

122 Ecotoxicity can be strongly affected by the mode of exposure. Dermal contact is  
123 commonly affecting an organism less than ingestion or interference with respiratory  
124 organs. This distinction is less relevant for, e.g., microorganisms and plants, but even  
125 for microorganisms and plants that have no intestines, internalization may occur and  
126 cause different toxicity than surface contact. In many cases, the nature of the  
127 organism's natural habitat and the test design will determine the mode of exposure.  
128 Plants may, e.g., be exposed in an aqueous suspension (seed germination tests and  
129 hydroponic plant cultures), or in solid matrices with more or less resemblance to a  
130 real soil at a site to be treated (El-Temsah and Joner 2012b). While exposure in  
131 aqueous suspensions may say something about the inherent toxicity of the  
132 nanoparticles tested, it will give a far higher exposure than equivalent tests using  
133 soil, and should thus include appropriate exposure estimates when questions of risks  
134 are addressed. When testing toxicity of nanoparticles to plants or soil organisms  
135 using soil as an exposure medium, the choice of a test soil is also decisive, as the  
136 relative amount of different soil constituents may vary considerably and affect both  
137 bioavailability of particles and whether plants or soil organisms thrive in them.  
138 Using a soil with a minimum of soil organic matter will go a long way to ensure  
139 that plants germinate and grow in them, or that earthworms are active in ingesting

soil during a test. But organic matter in soil may also result in a different availability of nanoparticles compared to a sub-soil void of humus, which is far more representative of soils being remediated using such particles. This is a trade-off situation where test organisms and test media should be selected as to be appropriate for the purpose of the test.

Another example of exposure control concerns earthworms. Dermal exposure of earthworms is usually measured by dissolving or suspending the material to be tested in water that imbibes a filter paper lining a glass vial where worms are placed (OECD 1984). Exposure through ingestion, on the other hand, uses a soil matrix where the material to be tested is mixed in. As in the example of exposing plant roots to nanoparticles in water or soil, exposure conditions for earthworms also differ greatly between water and soil. However, for worms the exposure matrix also determines mode of contact: nanoparticles suspended in water mainly result in dermal exposure, while nanoparticles mixed into soil or feed result in intestinal exposure plus dermal contact (Lapied et al. 2010). To relate data from the rapid and inexpensive dermal tests to test made with soil where bioavailability of nanoparticles is reduced by interactions with the soil components, one may perform dermal contact tests in soil by preventing worms from ingesting soil by gluing shut their mouths using super glue. The contribution from intestinal exposure may then be found by comparing worms with and without glued mouths.

For many test organisms, exposure through ingested material may differ widely according to how nanoparticles are introduced into the test system. Here, nanoparticles mixed into feed may result in far higher exposure than if directly mixed into soil. While certain earthworms (epigeic and anecic worms) seek out organic debris when they forage, other worms (endogeic worms) ingest soil and feed on the evenly distributed organic matter therein. Thus, exposing earthworms, e.g., to nanoparticles contained in organic feed or mixed homogeneously into soil that represents a volume that would frequently be at least 50 times higher may result in very different rates of uptake. Similarly, nematodes may be exposed to nanoparticles adsorbed onto bacteria upon which they feed, or through a suspension of nanoparticles where no prior association between bacteria and nanoparticles has occurred (Kleiven et al. 2018). To maximize ingestion by nematodes or other particle feeders (e.g., *Daphnia*), the test may omit the feed (e.g., bacteria and algae, respectively), but this may cause constipation and blockage of the digestive tract of the test organisms, and adverse outcomes that are caused by excessively high availability of nanoparticles (Roberts et al. 2007). In a more realistic exposure situation, the organisms would ingest mainly digestible particles that would ensure normal gut passage.

Aggregation (including agglomeration) of nanoparticles during exposure in aqueous media is a major determinant of exposure when particle uptake is size-dependent. Both medium constituents, particularly divalent ions like  $\text{Ca}^{2+}$ , excretions from test organisms and pH changes may cause this (Keller et al. 2012; Baker et al. 2014). Benthic organisms typically feeding on larger particles may potentially experience higher exposure due to aggregation, as discrete nanoparticles may be too small to be perceived as food. As mentioned above, surface-active compounds may

185 reduce aggregation and counteract aggregation effects, and even organisms may  
186 cause dispersion by producing organics that stabilize nanoparticles in suspension  
187 (Unrine et al. 2012).

### 188 28.3.1 *Endpoint Selection*

189 An ecotoxicological endpoint is the parameter measured as a response to a poten-  
190 tially toxic substance. Numerous endpoints may be used when assessing the effects  
191 on a test organism. For many organisms, mortality or growth rate are rather coarse  
192 endpoints used for testing acute toxicity, while enzymatic activities (e.g., of anti-  
193 oxidative enzymes), genetic mutations, or expression of genes related to damage  
194 repair or stress are gradually more sensitive test endpoints, permitting detection of  
195 more subtle and chronic adverse effects at lower concentrations (Walker et al. 2001).  
196 The interpretations of the responses to the endpoints with less obvious toxicity  
197 functions are however a minefield. Are they, e.g., altered behavior, avoidance,  
198 expression of stress-related genes, or enhanced frequencies of apoptosis indicators  
199 of toxicity? For example, if nZVI is introduced into soil or a test system, the redox  
200 conditions may change rapidly as to cause oxidative stress (or irreversible organis-  
201 mal damage) due to reactions of free O<sub>2</sub> with nZVI. But frequently such conditions  
202 are short-lived (El-Temseh et al. 2013), and oxygen will diffuse in from the border  
203 zones and re-establish oxygenated conditions and alleviate the stress caused by the  
204 reduced O<sub>2</sub> availability. For those organisms that have survived the period of  
205 reduced O<sub>2</sub> availability, the effects may be fully reversible, with no negative impacts  
206 on populations or communities (Nguyen et al. 2018a). Thus, if the exposure ceases  
207 and the organism has avoided it or only passed through a period with sub-optimal  
208 living conditions (stress) due to the nanoparticle exposure, it is not appropriate to  
209 interpret this as toxicity.

210 A particular consideration to make when it comes to testing of nZVI and other  
211 nanoparticles for remediation that may affect oxygen availability to organisms is the  
212 fact that these nanoparticles may cause a lack of oxygen needed by aerobic organ-  
213 isms during respiration. nZVI may, e.g., react with the available O<sub>2</sub> in the test  
214 medium as to render the conditions anoxic, thus asphyxiating the test organisms.  
215 This is particularly relevant for exposure in water and wet soil where O<sub>2</sub> diffusion  
216 and replenishment is slow. Such induction of anaerobic conditions and its detrimen-  
217 tal effects on aerobic organisms is not a nano-specific effect (though the dynamics of  
218 O<sub>2</sub> consumption may differ between nanoparticles and similarly reductive  
219 chemicals/bulk-size particles due to the specific surface area and chemical reactiv-  
220 ity). The effects nZVI may have on alternative electron acceptors (NO<sub>3</sub>, SO<sub>4</sub>,  
221 oxidized forms of Mn, etc.) can similarly preclude the use of anaerobic test organ-  
222 isms to circumvent the need for O<sub>2</sub> during testing.

223 Testing for nanoparticle-specific effects when the nanoparticles to be tested cause  
224 changes in the availability of O<sub>2</sub> or other electron acceptors require the use of control  
225 treatments that have comparable redox conditions, or the use of test systems or



exposure matrices that buffer against such changes, coupled with appropriate monitoring of redox potentials during the tests. 226  
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Nanoparticles that form colored suspensions may lead to a particular set of confounding effects related to shading of light. This is relevant for algae and other photosynthetic organisms that may experience lower light availability when used in tests where nanoparticle suspensions are dense enough to reduce transmittance (Handy et al. 2012b; Hjorth et al. 2016; Nguyen et al. 2018b). Algal growth rates or measurements of photosynthesis, chlorophyll content and related endpoints should thus account for confounding effects of shading. 228  
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**28.3.2 Trophic Interactions** 235

The trophic level of an organism can determine the way it is exposed to nanomaterials. This is partly due to the feeding habits of organisms at different trophic levels. Free-living microalgae may absorb nanoparticles directly from the water suspending the particles, but particles may also affect the algae by affecting the amount of available nutrient ions, or by shading the algae from light as to reduce photosynthesis. A filter feeder grazing on these algae may experience a similar concentration of nanomaterials through contact with water, but will in addition ingest, e.g., the aforementioned microalgae that may contain nanomaterials. Depending on whether bioaccumulation (increased concentrations in organisms with increasing lifetime) or biomagnification (predators accumulating higher concentrations than found in their prey) occurs, the next level predator may experience different exposure through the ingested food. So far, bioaccumulation has been observed for some nanomaterials (Petersen et al. 2008; Wang et al. 2013), and in some cases even biomagnification (Judy et al. 2011; Majumdar et al. 2016; Gupta et al. 2017). No such studies have been made with nanomaterials used for remediation. In some cases, the bioaccumulation may be due to the fact that an element contained in the nanoparticles in question is a micronutrient that the organism needs and scavenges for, as observed for cobalt nanoparticles (Coutris et al. 2012). Iron, found in many nanoparticles used for remediation, is likely to behave similarly if test organisms are experiencing sub-optimal iron supply. 236  
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**28.4 Standardized or Non-standardized Tests?** 256

A major part of the research on nanoparticle toxicity has been made using non-standardized tests, in the sense that they do not follow test protocols approved by standardization organizations like the Organisation for Economic Co-operation and Development (OECD) and International Organization for Standardization (ISO). Non-standardized tests have the advantage of choosing freely among organisms, endpoints, and exposure media. This allows for exposure optimization and 257  
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263 exploitation of the vast knowledge on biota, ranging from their behavior, physiol-  
264 ogy, metabolism, reproduction, and genetic peculiarities to community dynamics  
265 and ecosystem functions when interacting with their habitat. Non-standardized tests  
266 may thus be best suited to elucidate toxicity mechanisms or describe pertinent  
267 environmental consequences of spreading potentially toxic nanoparticles. In compar-  
268 ison, standardized tests are limited to easily culturable organisms exposed under  
269 well-defined conditions, using a limited number of rather crude endpoints. The  
270 advantages of using standardized tests are that the results can easily be compared  
271 with those obtained for other chemicals, which in turn permits hazard classification,  
272 and that standardized test results can easily be used for product documentation when  
273 chemicals are used in commercial products requiring approval regarding possible  
274 negative environmental effects.

#### 275 **28.4.1 Standardized Testing Methods**

276 OECD and ISO have published a number of test guidelines (TGs) that describe in all  
277 details how chemicals testing for approval of new chemicals should be conducted.  
278 These tests have been developed for soluble chemicals and have not taken into  
279 account considerations that may be important for toxicity testing of nanoparticles.  
280 Yet, the OECD has concluded that the approaches for testing and assessment of  
281 traditional chemicals are in general appropriate for assessing nanomaterials, but that  
282 the tests may have to be adapted to the specificities of nanomaterials (see ref.  
283 [OECD](#)). This concerns, e.g., methods of sample preparation, particularly regarding  
284 homogenization and distribution of the nanoparticles in the test media. Similarly,  
285 adaptations may be needed for certain test guidelines.

286 The first step is the preparation of a stable suspension of nanomaterials (see  
287 references in [Hund-Rinke et al. 2016](#)). This can be obtained through the use of  
288 surface-active agents reducing the attractive forces between particles and causing  
289 them to remain suspended in water or other media for a period of time that would  
290 permit absorption or other interactions causing harm to the test organism. These  
291 surfactants may themselves affect the test organisms, and thereby the test outcome,  
292 and should therefore be included in control treatments.

293 It is a common requirement of standardized test guidelines that exposure con-  
294 centrations should remain stable (often stated as no more than 20% deviation  
295 between exposure concentrations and nominal concentrations) over the duration of  
296 the test. This is a challenge with nanomaterials, which easily agglomerate and  
297 sediment out of a water column (to mention the case of aquatic tests), exposing  
298 pelagic organisms to lower concentrations and benthic organisms to higher concen-  
299 trations than originally intended. Several factors influence the agglomerating and  
300 settling behavior of particles, such as agitation of the test system, ionic strength, pH,  
301 presence of specific ligands/chelating agents, and organic matter content of the

exposure medium. The following modifications have been proposed by Hund-Rinke et al. (2016) for maintaining (more) constant exposure conditions in test systems:

- Conduct the OECD TG 202—acute immobilization of *Daphnia magna* test (OECD 2004) at pH values enabling more stable dispersions of nanomaterials and use a growth medium with very low ionic strength, e.g., very soft EPA medium. This medium has been shown to allow normal growth and reproduction of *D. magna*.
- In the OECD TG 210—fish, early-life stage toxicity test (OECD 2013) with zebrafish, improve nanomaterial dispersion by using exposure chambers coupled with water changes every 24 h.
- In tests using spiked soil or sediment, i.e., OECD TG 216—nitrogen transformation test (OECD 2000a); OECD TG 217—carbon transformation test (OECD 2000b); OECD TG 220—enchytraeid reproduction test (OECD 2016a); OECD TG 222—earthworm reproduction test (*Eisenia fetida*/*Eisenia andrei*) (OECD 2016b); OECD TG 225—sediment-water lumbriculus toxicity test (OECD 2007), add nanomaterials to each replicate, to ensure homogeneity of spiking. Exceptions can be made for low concentrations, where this modification can be difficult to implement.

There has been a concern that some OECD test guidelines were not suited for the detection of toxic effects, in the sense that they would underestimate the potential toxicity of some nanomaterials. One way this underestimation could occur is by reduction of the bioavailability of nanomaterials and their transformation products due to sorption to organic matter or the elevated pH. Underestimation of the toxicity of nanomaterials can also occur when the duration of the test is too short compared to the slow transformation of nanomaterials in soil, which can be the source of toxic chemical species. The following modifications proposed by (Hund-Rinke et al. 2016) may minimize the interference of the nanomaterials with the components of the test media or the toxicity endpoints:

- OECD TG 201—freshwater alga and cyanobacteria, growth inhibition test (OECD 2011): the chelating agent EDTA can interfere with metal nanomaterials and a modified EDTA-free version of the OECD algal medium (OECD-M) for *Raphidocelis subcapitata* is proposed.
- For the OECD TG 201 (OECD 2011), it is recommended to measure biomass by determination of in vitro chlorophyll a, instead of optical density and in vivo fluorescence measurements or cell counting by hemocytometry.
- OECD TG 216—nitrogen transformation test (OECD 2000a): for the testing of ion-releasing metal nanomaterials, the pH of the soils should be at the lower end of the range accepted according to the test guideline (pH 5.5). It is also proposed to extend the duration of the test to 56 days, since some nanomaterials only show effects after ageing, and to include multiple short-term measurements of the potential ammonium activity, instead of single measurements at the start and the end of the test.

344 • OECD TG 217—carbon transformation test (OECD 2000b): similar modifica-  
345 tions are proposed for this test, except the multiple short-term measurements.

#### 346 **28.4.2 *Fe-Based Nanoparticles Exempt from Nano-Fear***

347 nZVI and Fe-oxide-based nanoparticles have, to some extent, dodged the skepticism  
348 that clings to other types of nanoparticles. This is partly because nanoparticles for  
349 remediation are used to treat and remove the harmful effects of toxic pollutants, thus  
350 reducing the exposure of humans and the environment to highly toxic and mobile  
351 chemicals like TCE (trichloroethylene) for which there are no doubts of adverse  
352 effects or environmental exposure. Further, Fe-based nanoparticles have repeatedly  
353 been shown to have limited or even very limited mobility in the environment,  
354 restricting movement away from the treated areas (Johnson et al. 2013), which are  
355 often fenced in and unavailable to the public. Thus, risks appear confined to the  
356 treated areas. A third point in favor of Fe-based nanoparticles comes from the fact  
357 that most natural environments contain ample amounts of Fe, even in forms similar  
358 to those coming out of nanoremediation treatments. The products of nZVI aged in  
359 aerated water are mainly  $\text{Fe}_3\text{O}_4$  (magnetite) and  $\gamma\text{-Fe}_2\text{O}_3$  (maghemite), accompanied  
360 by  $\gamma\text{-FeOOH}$  (goethite). If corrosion continues, the products are predominantly  
361  $\gamma\text{-FeOOH}$ , with small amounts of  $\text{Fe}_3\text{O}_4$  and  $\gamma\text{-Fe}_2\text{O}_3$  (Liu et al. 2015). The final  
362 aqueous corrosion product of nZVI is FeOOH (Pullin et al. 2017; Lei et al. 2018).  
363 Finally, Fe-based nanoparticles have been scrutinized in several research projects in  
364 parallel to their development, and the outcome of the ecotoxicity measurements as  
365 well as practitioners feedbacks indicate that Fe-based nanoparticles are causing low  
366 concern, if any (Bardos et al. 2011; Hjorth et al. 2017).

#### 367 **28.4.3 *Ecotoxicity Does Not Equal Risk***

368 Risk is the product of hazard (ecotoxicity) multiplied by the probability of encoun-  
369 tering hazard (exposure). As mobility of nZVI and other Fe-based nanoparticles is  
370 limited, and as their prescribed use targets subsoils at several meters depth in  
371 industrial brownfields, the risk to humans (apart from workers exposed during  
372 production, transport, and deployment) and wildlife is extremely low or inexistent.  
373 The low hazard level due to the low inherent toxicity of the Fe-based nanomaterials  
374 of course also contributes to the low risks.

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