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Environmental impact of engineered carbon nanoparticles: from releases to effects on the aquatic biota

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Laury Gauthier^{1,2} and Emmanuel Flahaut^{3,4}

Nano-ecotoxicology is an emerging science which aims to assess the environmental effect of nanotechnologies. The development of this particular aspect of ecotoxicology was made necessary in order to evaluate the potential impact of recently produced and used materials: nanoparticles (NPs). Among all the types of NPs, carbon nanoparticles (CNPs) especially draw attention giving the increasing number of applications and integration into consumer products. However the potential impacts of CNPs in the environment remain poorly known. This review aims to point out the critical issues and aspects that will govern the toxicity of CNPs in the environment.

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Introduction

Nanoparticles (NPs) are usually defined as objects with at least one dimension between 1 and 100 nm. They can be released into the environment from natural (volcanoes, forest fires, *etc.*) or anthropogenic (brake pads residues, welding, combustion, *etc.*) sources. Among anthropogenic nanoparticles, engineered nanoparticles (ENPs) have recently emerged and quickly shown a very fast development [1]. The fields of applications of ENPs are many (automobile, medicine, optics, electronics, *etc.*) and ENPs are now integrated in daily life consumer products. The

number of products integrating nanoparticles was estimated between 1814 [2*] and 2332 [3] and was in 2015 30-fold more important than in 2005 [2*]. Among the wide variety of ENPs, Carbon-based nanoparticles (CNPs) represent a specific class, especially interesting in terms of rapid development and applications. Although all composed of carbon atoms, the different hybridization of the C–C bonds gives them very specific physical properties. CNPs can be distinguished between 0D: fullerenes, onion-like carbon, carbon dots, nanodiamonds; 1D: nanofibers, nanotubes and nanohorns; 2D: multilayer graphitic nanosheets, graphene nanoribbons, and graphene and related materials (GRMs). CNPs were first described in 1985 with C₆₀ fullerene [4] but most applications came later with carbon nanotubes (CNTs) in 1991 [5] and graphene more recently [6]. Given their unique properties, GRMs are currently subject to important research efforts to improve their large scale production [7,8]. CNPs are already used in daily life products (nanocomposites, paints, energy storage, waste water treatment [9], *etc.*). Depending on data sources, between 89 and 217 consumer products integrate carbonaceous nanomaterials [2*,3] and it is likely that CNPs will be released in the environment during the life cycle of manufactured products [9–11]. This review aims to report the state of the art dealing with CNPs effects on the environment with a special focus on the aquatic environment because of its ability to concentrate pollution. An emphasis will be made on the fate and detection of CNPs in the environment and in complex biological matrices.

Carbon nanoparticles in the environment

The risk posed by a xenobiotic in the environment is defined as the result of environmental exposure and its intrinsic danger. The releases and fate will govern concentrations of CNPs in the environment and are thus key aspects that will determine their ecotoxicity.

Analytical measurements of CNPs in complex matrix

Several experimental techniques are currently used and developed in order to directly measure environmental concentrations of CNPs. Most types of nanoparticles such as metal nanoparticles can be more easily detected and quantified in complex organic matrix (especially using single particles inductively-coupled plasma quadrupole mass spectrometer: sp-ICP-MS or synchrotron) [12].

However, the intrinsic nature of CNPs but also many technological barriers prevent their reliable detection in carbon-rich complex environmental matrices: quantification of CNPs is often more difficult than looking for a needle in a hay stack. Among CNPs, a real effort was put on the detection of CNTs. Recent reviews [13^{**},14,15,16] identified available technologies for extraction (a dozen) and measurement (around twenty) of CNPs in both the environment and in organisms but also highlighted all the limitations of these techniques. The lack of hindsight concerning the robustness of these methods but also the lack of reproducibility is pointed out. However thermal methods such as microwave-induced heating (MIH) [17] or PTA (programmed thermal analysis) seem promising and have also been successfully used to measure graphene and graphene oxide in complex organic matrices [18].

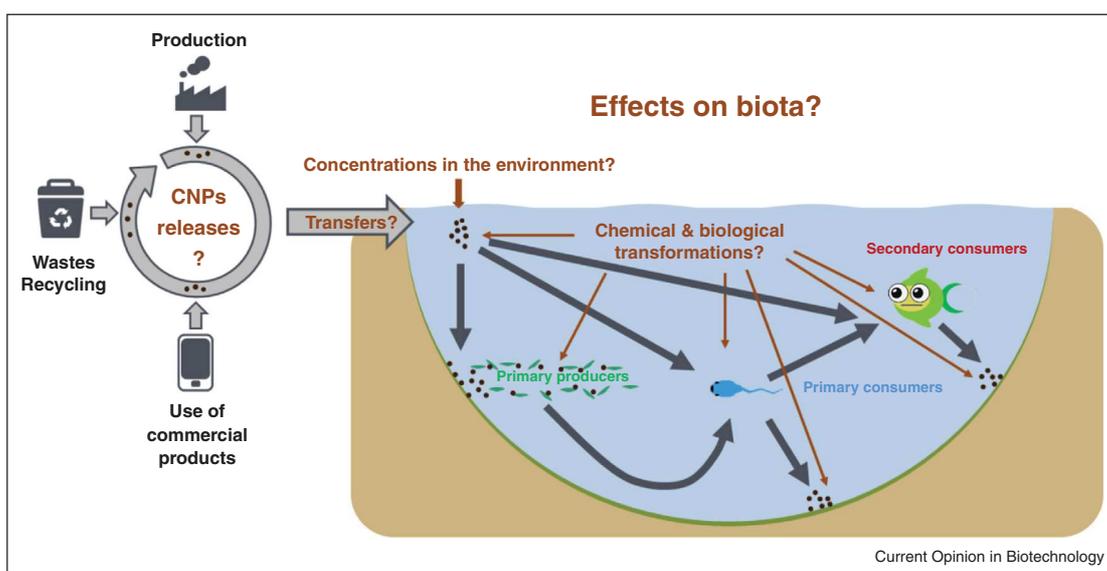
Release and fate

Release of CNPs into the environment could occur at each stage of the life cycle of manufactured nano-products: production, use, waste, and disposal [9,10,19] (Figure 1). Without reliable and robust analytical methods for detecting trace concentrations of CNPs (apart from the special case of isotopic labeling with ¹³C and ¹⁴C), mathematical modeling is a useful tool to predict releases and environmental concentrations. Studies modeling the CNPs release mainly focused on CNTs as well as graphene more recently, and few information is available for other types of CNPs [11,19,20^{**},21]. Available data showed that worldwide production of CNTs is close to 3 kt/year [10] and European production contributes about 0.38 kt/year [19]. Based on European production data, latest estimations

reported environmental predicted concentrations (EPCs) in surface water between 0.23 ng/L ($Q_{0.15} = 0.17$ ng/L; $Q_{0.85} = 0.35$ ng/L) in 2012 [19] and 0.28 ng/L ($Q_{0.15} = 0.04$ ng/L; $Q_{0.85} = 0.65$ ng/L) in 2014 [20^{**}]. Similarly sediment concentrations were estimated and ranged from 0.79 ng/L ($Q_{0.15} = 0.61$ ng/L; $Q_{0.85} = 1.2$ ng/L) in 2012 [19] to 6.34 ng/L ($Q_{0.15} = 4.32$ ng/L; $Q_{0.85} = 9.24$ ng/L) in 2014 [20^{**}]. With an expected continuous increase in needs, the concentration of CNPs will increase in all environmental compartments. Although very useful, these predictions are not validated by analytical measurements [22] and improvements are needed in both fields [23].

Modeling methods are also used to determine the fate of engineered CNPs [24], which is governed by both biotic and abiotic processes [25]. These transformations could drastically change the behavior and the bioavailability of CNPs [9,15,26]. The review by Mitrano *et al.* gives a complete overview of the aging and transformations that CNTs may experience in the environment and during the life cycle of manufactured products [9]. Because of transformations, the behavior and physical properties of pristine CNPs might be completely different following their release. A complete characterization of nanomaterials has become a requirement to publish nanotoxicological data. There is however some ambivalence between the need of full characterization of pristine CNPs (just manufactured) and transformations of these particles after interaction with exposure media and organisms during ecotoxicological trials. Physicochemical characteristics are necessary to understand toxicological phenomena but, as reliable analytical measurements and detection of CNPs in complex

Figure 1



Schematic representation of the potential pathways and contamination of the environment by CNPs.

matrices are still improving and mostly do not allow characterization afterwards, the need of characterization data of pristine materials sometimes sounds pointless. Transformations and aging of CNPs are challenging research topics but they are of fundamental importance in order to realistically assess the effects of CNPs in complex environments (Figure 1).

Assessment of CNPs toxicity in the biota

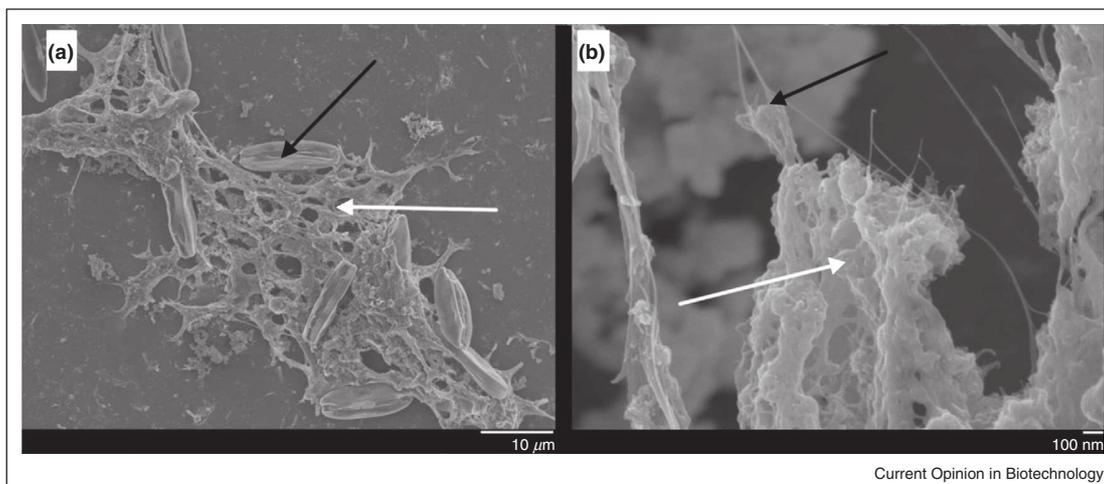
Historically, assessment of CNPs ecotoxicological effects relied upon methodologies used for 'classical contaminants' (*i.e.* chemicals). For 'new' contaminants such as CNPs, these test methods have initially played a role in order to define toxicity thresholds. Due to recent developments and uses, recent studies on CNPs' ecotoxicological potential mainly focused on the effects of CNTs, graphene and GRMs.

Photosynthetic microorganisms are at the base of many trophic chains. Toxic effects on these micro-organisms could lead to drastic effects on the whole trophic chain (Figure 1). These organisms are thus a critical group to look at for assessing the effects of CNPs on the environment. Effects of two different types of CNTs (double walled: DWCNTs and multiwalled: MWCNTs) were assessed on the benthic diatom *Nitzschia palea* [27,28]. Results showed that environmentally realistic concentrations of natural organic matter (NOM) used as a dispersant could increase the short term growth inhibition induced by CNTs. Dispersion of CNPs is essential to characterize as it will greatly determine the bioavailability of these particles. A second essential issue concerns the secretion of extracellular polymeric substances (EPS) which has a protective role against CNTs and helps for growth recovery (Figure 2). Furthermore the EPS-coated

CNTs could potentially move to higher trophic levels (Figures 1 and 2) of the food chain, after being grazed by organisms. Oxidized nanomaterials (carboxylic function-containing single walled CNTs: C-SWCNTs and graphene oxide: GO) exhibit a different toxicological profile with generation of reactive oxygen species (ROS) from 0.01 mg/L in the green algae *Chlorella vulgaris* [29]. Oxidative damages were also detected in the protozoa *Euglena gracilis* exposed to GO [30]. CNPs effects on photosynthetic organisms also depend on the intrinsic nature of the CNPs and on the physiology and anatomy of these organisms. For instance, oxidized particles appear more toxic and are associated with oxidative stress. Some characteristics, such as the presence of the cellular wall or the secretion of EPS seem to make algae more resistant [27–29] compared to other organisms [30]. The question of the role of oxygen-containing functions is still debated because not only this modifies the surface chemistry on the CNPs and thus their surface charge depending on the pH, but it also contributes to make them much easier to disperse in water.

Amphibian models such as *Xenopus laevis* are well characterized (genetics, development and physiology) and are very relevant candidates for ecotoxicology assessment. *X. laevis* was used to assess genotoxicity and toxicity of CNPs using standardized procedures [31–33]. If oxidative stress and DNA damages (repairable) were evidenced after short term exposure, CNTs exhibited no genotoxic potential since no micronuclei (thus non-repairable damages) were observed after a 12-day exposure. However growth inhibition was observed at high concentrations (from 10 mg/L). Growth is a crucial parameter whose measurement integrates all modifications and disturbances undergone by an organism. Based on

Figure 2



(a) Scanning electron microscopy images of *Nitzschia palea* (dark arrow) with extracellular polymeric substances (white arrow) after exposure to DWCNTs. (b) Scanning electron microscopy images of exopolymeric substances (EPS) (white arrow) and CNTs embedded by EPS (dark arrow).

publications in the field of respiratory nano-toxicology [34] we recently evidenced that surface area of CNPs (DWCNTs, MWCNTs, nanodiamonds and few layer graphene) is likely to be the main parameter determining the growth inhibition in *X. laevis* larvae [35]. A special focus should be made in the future on the use of non-classical dose metrics which could allow uncovering some toxicological processes because mass-based concentration is generally not appropriate.

Toxicity data of CNPs in single species tests could be gathered in order to mathematically model predicted no effect concentrations (PNECs) for the whole biota. Using probabilistic species sensitivity distribution (pSSD), Coll *et al.* derived PNECs for CNTs and fullerenes [36]. The PNECs values for these two CNPs were respectively 55.6 µg/L (39.9–78.0) and 3.84 µg/L (2.7–5.3). Using the same type of approach (SSD), HC₅ (predicted concentration at which 5% of species are harmed) values were calculated for fullerenes and CNTs and ranged between 0.2 and 5 mg/L, which is also far above PECs [37]. To date, PNECs values are far more important than PECs and based on these approaches, environmental effects of CNPs seem limited.

Transformations and aging are key points to fully assess the environmental impact of CNPs. Classical ecotoxicological studies using single-species (*i.e.* normalized test) could help to understand toxicity mechanisms or effect on a particular taxa. However there is a lack of environmental relevance using such tests and critical parameters such as biotransformation, bioaccumulation, biomagnification or effects of abiotic factors are ignored. Complex exposure systems are thus increasingly used to assess the effects of NPs [38]. The range from indoor trophic chains and multispecies exposures to outdoor micro and mesocosms exposures [39]. These studies allow experimental conditions closer to the real ecosystem however with less control of both biotic and abiotic parameters. These complex systems were used to assess the effects of various types of nanomaterials [40–43] but only one publication is available for CNPs [44], revealing the low mobility and bioavailability of CNTs in aquatic ecosystems, whereas high persistence in sediment was observed.

Conclusions

Mathematical modeling studies have shown that predicted environmental concentrations are expected to be quite low and typically close to 1 ng/L. Since applications and production of CNPs are increasing, it is expected that environmental concentrations will also increase. The biggest challenges concern the direct measurement of CNPs in complex environmental matrices in order to validate calculated concentrations. Progresses in both modeling and analytical measurement are needed for a trustworthy assessment of the environmental impact of CNPs.

Classical monospecific tests reveal toxic effects at concentrations far higher than the predicted ones. However several limitations should be taken into account suggesting that the potential impact may be higher than expectable. The use of the classical approach of mass-based concentration as the favorite metrics to express and compare toxicity results should evolve, and surface-based concentration should be seriously considered instead, especially in the case of CNPs. Ecotoxicology should move from the classical ‘toxicology’ approach toward a more relevant ‘eco’ evaluation of CNPs’ impacts. Identification of specific toxic effects remains unavoidable to understand the mechanisms of intoxication of living systems but these studies must be completed with more complex but also more realistic exposures reflecting the real environment. It may allow to uncover toxicity at levels where no effects could be observed until now with simpler exposure methods like the classical single species test systems [41]. Finally we should go toward the use of integrated biomarkers and approaches (*i.e.* biodegradation, growth) reflecting all the disturbances induced by CNPs at lower levels of organization. This will give clearer responses for the environmental risks posed by CNPs, and nanoparticles in general.

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Conflict of interest

The authors declare no competing financial interest.

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This mathematical modeling study provides the most recent assessment to date concerning the concentrations of CNTs in different environmental compartment. As direct detection of CNPs by analytical tools still need improvement, this study allows a predicted overview of the potential concentrations in water. Combined with sensitivity assessment of organisms to CNPs, the data provided in this study are thus very valuable for environmental risk assessment of CNPs.

By using a methodology previously described in aerial nanotoxicology studies, this research article highlighted the importance of using "non-classical" dose metrics for studying the ecotoxicological effects of CNPs. The authors evidenced using an amphibian model that the toxicity of different kinds CNPs is mainly governed by surface area. Moreover the different types of CNPs could be considered as one when their toxic

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