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# A preliminary investigation on nanohorn toxicity in marine mussels and polychaetes☆

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## HIGHLIGHTS

- This is the first attempt to evaluate nanohorn toxicity in model marine invertebrates.
- Mussels and polychaetes were exposed to three nanohorn concentrations: 1, 5, and 10 mg $L^{-1}$.
- Acute effects were evaluated at 24 and 48 h using biochemical and lysosomal biomarkers.
- Nanohorns induced sub-lethal effects to antioxidant system and lysosomes in mussels.
- Polychaetes were partially responsive to oxidative stress induced by nanohorns.

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## ABSTRACT

Single walled carbon nanohorns (SWCNHs) are a black nanoscale spherical aggregate of cylindrical tubes of graphitic carbon which differ from nanotubes in their "horn-like" shape. Their peculiar structure makes them one of the best electronconductors at a nanoscale level. Although not commercially exploited, their rapid environmental diffusion is expected to rise significantly in the next few years. Therefore, we appraised the ecotoxicology of SWCNH powders by taking into account the ecological role of the two species that were deployed in exposure experiments: polychaetes, *Hediste diversicolor*, and mussels, *Mytilus galloprovincialis*. Adult mussels and polychaetes were exposed to three SWCNH concentrations: 1, 5, and 10 mg $L^{-1}$ and acute effects were measured after 24 and 48 h. Sub-lethal effects were estimated at level of physiological functions such as digestion in mussels (i.e. variations in lysosomal parameters and lipofuscin content) and the antioxidant system in both species (i.e. glutathione peroxidase activity and malondialdehyde content). SWCNH suspension in sea water was also characterised, highlighting the formation of aggregates the size of which was related to SWCNH concentrations and their resident time in the medium. The results showed that SWCNH affected the oxidative and lysosomal systems on the hepatopancreas and led to lysosomal alterations on haemocytes in mussels. The biological responses were less clear in polychaetes. This preliminary investigation highlighted the need of focusing future research efforts on possible physiological impairments caused by long-term exposure to SWCNHs in marine species.


## 1. Introduction

The development and use of nanomaterials (NMs) are growing at an ever accelerating rate in many industrial areas (e.g., materials science, personal care products, electronics and, more recently, medicine), due to their exceptional physico-chemical properties, such as the large surface area to volume ratio, their great reactivity and quantum effects (Farré et al., 2011; Pérez et al., 2009). NMs, consisting of natural, incidental and/or engineered particles, are defined as materials consisting of nanostructures with at least one dimension less than 100 nm, e.g. nanofilms and nanocoatings, and with two/three dimensions at the nanoscale, e.g. nanotubules, nanowires, and nanoparticles (NPs), respectively (Moore, 2006). Engineered NPs can be classified into four groups: carbon NMs, metal-based NPs, quantum dots and dendrimers. Because of their widespread use in various consumer products, it is expected that these materials will find their way into the environment; as a consequence, the potential ecotoxicological risk associated with NMs has recently aroused growing interest, particularly as regards the occurrence, behaviour and fate of engineered NPs in the coastal marine environment which is expected to be their ultimate sink, as is for almost all the other anthropogenic contaminants (Klaine et al., 2008; Zhao and Liu, 2012). Notwithstanding the growing

☆ Capsule: Biological responses at biochemical and lysosomal levels are affected by nanohorn exposure both in mussels and polychaetes.

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understanding for the potential ecological hazard, the toxicity of NMs to environmentally relevant marine/estuarine species has started to be investigated only recently (Canesi et al., 2012; Cattaneo et al., 2009). Moreover, most of the available information about their environmental fate and ecotoxicological effects is limited to few types of NPs, mainly carbon nanotubes, fullerenes and silver and metal–oxide NPs (Bondarenko et al., 2013; Buffet et al., 2013; Kadar et al., 2012).

SWCNHs are horn-shaped aggregates of tubular structures (diameter 3–4 nm, length 20–40 nm) with the apex capped with five pentagonal carbon rings. As single-walled nanotubes (SWNTs), fullerenes and graphene, the geometry of the nanohorn (i.e. size, surface area, shape, solubility, aggregation) defines a unique set of structural, mechanical and chemical properties. Although individual SWCNH structure is similar to individual nanotubes, SWCNHs typically tend to assemble forming rough surface spherical aggregates (i.e. dahlia, buds and seeds), with overall diameters of about 60–100 nm, which, unlike the bundle aggregates of SWNTs, give them a large surface area and seem to be useful for holding other materials, keeping their particle size small. Although at present SWCNHs are not commercially exploited, they are considered promising materials offering a wide range of potential applications in the catalysis, storage and delivery of adsorbed substances, such as gas adsorption, biosensing, drug delivery, gas storage and catalyst support for fuel cell. Moreover, their high yield and purity deriving from the production method without typical metal catalysts, like iron, nickel or cobalt, are all features which have facilitated the development of potential applications in the absorption and release of small molecules (Lynch et al., 2007; Yudasaka et al., 2008).

The main objective of this study was to provide a first insight into the likely toxicity associated with single-walled carbon nanohorns (SWCNHs) dispersed in natural sea water, by evaluating their potential acute effects on two estuarine invertebrate organisms, mussels and polychaetes. The acute effects of SWCNHs have been investigated in vivo on the filter-feeding bivalve mollusc *Mytilus galloprovincialis* and on the benthic estuarine worm *Hediste diversicolor*, exposed to contaminated water in static aquaria. These two species were selected because of their key role in the functioning of estuarine coastal ecosystem, the former being commonly indicated as a keystone species for benthic food-web structure and macroinvertebrate community, the latter affecting the biogeochemical cycles of nutrients and pollutants and also being a main food source for estuarine fish and birds (Mouneyrac et al., 2010). Moreover, they are widely accepted as good biomonitor organisms; mussels, in particular, have been used for a long time as "warning sentinels" because of their early and good responsiveness to various anthropogenic disturbances (Goldberg et al., 1978). More recently, also the use of polychaete worms as bioindicators for estuarine ecosystems has gained good evidences to be a useful tool in the assessment of environmental quality, being not insensitive to stressful environmental conditions (Dean, 2008).

In this study we have investigated some biochemical changes in the antioxidant system and other cellular alterations in histochemical parameters which may indicate impairment in the lysosomal functions, after considering the available scientific information, which supports the hypothesis that both immune cells and digestive tissues may be major targets for NM toxicity in marine invertebrates, especially molluscs (Ciacci et al., 2012; Couleau et al., 2012; Koehler et al., 2008). The antioxidant enzyme activity was evaluated by determining glutathione peroxidase (GPx), and malondialdehyde (MDA) contents in both mussels and polychaetes. Lysosomal biomarkers, i.e. lysosomal membrane stability (LMS), lysosomal structural changes (LSCs) and lipofuscin accumulation were determined in the digestive glands of mussels. LMS was also evaluated in mussel haemocytes by the neutral red retention (NRR) assay. Lastly, a qualitative histological assessment of mussel digestive glands was also performed, aiming for a better comprehension of the histochemical and biochemical data.

## 2. Materials and methods

### 2.1. Nanofluid preparation and particle size measurements

The nanomaterials to be tested were kindly provided by the private company Carbonium Srl, (Padova, Italy) and consisted in pure SWCNHs prepared by a new patented process (Pagura et al., 2012). Briefly, the method is based on heating of graphite rods by induction of very intense, high frequency, eddy currents. In the same paper, detailed chemico-physical and morphological characteristics of SWCNHs are reported. The morphology of SWCNHs evaluated by transmission electron microscopy (TEM — FEI Tecnai 12) and field emission SEM (FE-SEM — Zeiss ΣIGMA) is shown in Fig. 1.

The experimental SWCNH dispersions were set according to the following procedure: the SWCNHs were firstly mechanically dispersed in deionized water to obtain a 0.1 wt % stock solution, subsequently homogenised through an ultrasonic processor (VCX 130, Sonics & Materials) at 20 kHz and 70 W for 10 min to assure long term stability to the dispersion; particle size measured before and after the homogenization process revealed a mean value of about $70 \pm 10$ nm. The stock solution was then diluted in 1 μm-filtered natural sea water at 33 (FSW) to achieve the needed concentrations (1, 5 and 10 mg $L^{-1}$). Measurements of the average size of SWCNHs in water samples taken from the experimental tanks before, during and at the end of the experiments were performed through a Zetasizer Nano ZS (Malvern). This instrument calculates the particle size (in the range 0.6 nm–6 μm) according to the Stokes–Einstein equation (Pecora, 1985), measuring

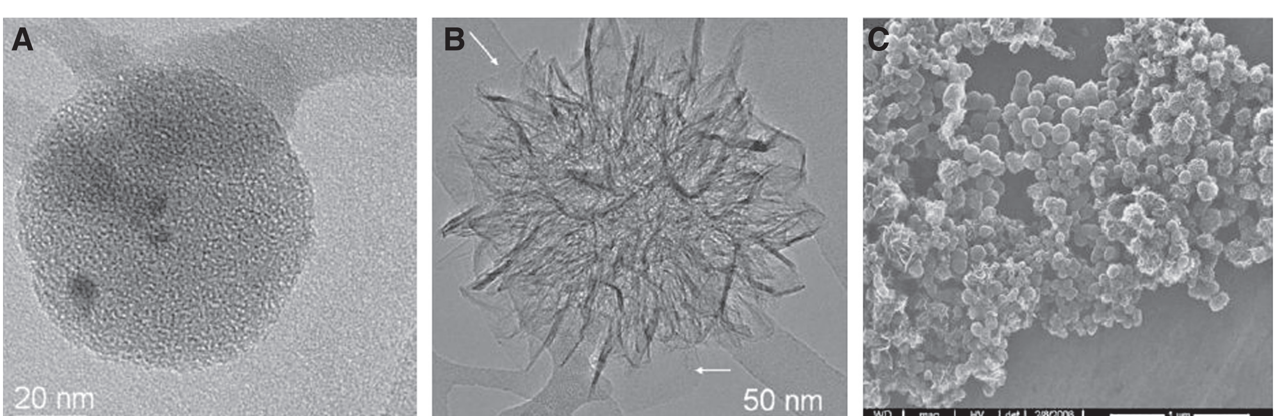


**Fig. 1.** A and B: TEM images of single nanohorn (bud-like and dahlia-like, respectively). Tiny foils of graphene are indicated by the white arrows. C: images of bud-like and dahlia-like obtained with FE-SEM.
From Pagura et al. (2012).