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# Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells

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## ABSTRACT

Plastic wastes are among the major inputs of detritus into aquatic ecosystems. Also, during recent years the increasing use of new materials such as nanomaterials (NMs) in industrial and household applications has contributed to the complexity of waste mixtures in aquatic systems. The current effects and the synergism and antagonisms of mixtures of microplastics (MPLs), NMs and organic compounds on the environment and in human health have, to date, not been well understood but instead they are a cause for general concern.

The aim of this work is to contribute to a better understanding of the cytotoxicity of NMs and microplastics/nanoplastics (MPLs/NPLs), at cell level in terms of oxidative stress (evaluating Reactive Oxygen Species effect) and cell viability. Firstly, the individual cytotoxicity of metal nanoparticles (NPs) (AgNPs and AuNPs), of metal oxide NPs ($ZrO_2$NPs, $CeO_2$NPs, $TiO_2$NPs, and $Al_2O_3$NPs), carbon nanomaterials ($C_{60}$fullerene, graphene), and MPLs of polyethylene (PE) and polystyrene (PS) has been evaluated *in vitro*. Two different cellular lines T98G and HeLa, cerebral and epithelial human cells, respectively, were employed. The cells were exposed during 24–48 h to different levels of contaminants, from 10 ng/mL to 10 µg/mL, under the same conditions. Secondly, the synergistic and antagonistic relationships between fullerenes and other organic contaminants, including an organophosphate insecticide (malathion), a surfactant (sodium dodecylbenzenesulfonate) and a plasticiser (diethyl phthalate) were assessed. The obtained results confirm that oxidative stress is one of the mechanisms of cytotoxicity at cell level, as has been observed for both cell lines and contributes to the current knowledge of the effects of NMs and MPLs-NPLs.

## 1. Introduction

During the last century, technological developments triggered the promotion of new materials such as plastics which, due to their excellent mechanical properties, versatility and low cost impelled their consumption. Therefore, since the 1940s, when the first production of plastics at the industrial scale took place, their manufacture has steadily grown (Al-Salem et al., 2010). In addition, from 2000 to 2010 a massive growth of the world's yearly consumption was produced in relation to the growing demand in developed economies such as the EU, Canada and the USA, together with the new demand and enhanced consumerism in emerging economies such as China. However, this expansion comes with the generation of tonnes of waste, some of which are non-biodegradable and toxic by-products, resulting in new undesirable impacts on the environment (Tongesayi and Tongesayi, 2016). Moreover, to date, the environmental fate and time of total degradation have not been well understood. In particular, microplastics (MPLs), defined as small particles at the millimetre to sub-millimetre size range with high densities, are a new environmental risk (da Costa et al., 2017; Horton et al., 2016). The origin of MPLs can be from the manufacturing of micro-beads that are used in cosmetic facial cleansers (Fendall and Sewell, 2009). Alternatively, secondary MPLs can result from the fragmentation and erosion of plastic items (Aueviriyavit et al., 2014). During recent years, the presence of plastics and MPLs in marine and coastal areas has been a cause for concern (Cole et al., 2011), attracting the attention of both researchers and the general public. Low density MPLs contaminate the sea surface and due to their small size, which is similar to plankton, they could be introduced into the food chain (Wright et al., 2013). Another important uncertainty is whether these compounds can act as vector for transferring pollutants to biota,

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thus leading to their bioaccumulation (Llorca et al., 2014; Cedervall et al., 2012).

On the other hand, during the last decade, developing nanotechnology has promoted a new series of materials and opportunities for different industrial and consumer products. Nanomaterials (NMs) are characterized by their dimensions in the range of 1–100 nm. Key factors driving the fact that NMs differ significantly from pollutants at the macro-scale are their high surface area and quantum effects (Farré and Barceló, 2012). These factors can modify some properties such as electrical or optical reactivity and strength, thereby altering the fundamental physical and chemical properties of conventional materials. Current applications in nanotechnology span from electronics (Sanchez et al., 2011), household products, food additives and food packaging (Eleftheriadou et al., 2017; Ranjan et al., 2014), personal care products, nanomedicine (Marchesan and Prato, 2013), sports equipment, textiles (Contado, 2015), among many others. With the ongoing commercialization of NMs, human exposure to nanoparticles will dramatically increase, and evaluation of their potential toxicity is essential (Holden et al., 2016).

During recent years, a high number of studies have evaluated the ecotoxicological effects on NMs (Yan et al., 2011; Fu et al., 2014) as well as the environmental fate and behaviour of NPs (Nickel et al., 2014). In particular, NMs and NPs, which currently have an extensive range of applications such as metal and metal oxide NPs (among which there are silver nanoparticles (AgNPs), gold nanoparticles (AuNPs), and titanium dioxide nanoparticles ($TiO_2$), and more) have been studied more by using *in vivo* and *in vitro* approaches (Johnston et al., 2010; Wijnhoven et al., 2009). For instance, due to the antibacterial properties of AgNPs, they are exploited in an increasing number of consumer and medical products such as packaging materials (Aueviriyavit et al., 2014), clothing, wound dressings, deodorants and spray rooms (Vigneshwaran et al., 2007; Lee et al., 2007). Regarding Au-NPs, they are used in current biomedical applications including diagnostics, photo-thermal and photodynamic therapies and delivery of target molecules such as drugs or peptides (Austin et al., 2015; Dykman and Khlebtsov, 2012). On the other hand, recent research has investigated the uptake in plants of some metal oxide NPs such as $nCeO_2$ (nanoscaled cerium oxide) and the introduction of these into the food chain (Rico et al., 2011). On the other hand, other NMs such as fullerenes and carbon nanotubes (CNTs) have recently been considered in different toxicological and bioaccumulation studies (Maes et al., 2014) because, firstly, in addition to nanotechnology they can be emitted by incidental and natural sources into the environment. Secondly, because of their adsorption/desorption capabilities, they could influence the toxicity of other co-contaminants (Sanchís et al., 2016).

The lack of information on the toxicology of NMs under certain scenarios of exposure has led to the restricted use of some of them for certain applications that are directly in contact with humans, such as inclusion in cosmetics, detergents and food, in the prevention of their potential toxicity or long-term secondary adverse effects (Yoo-Iam et al., 2014; Khanna et al., 2015). But, due to the impact of anthropogenic activities, the concentrations of some NPs in the aquatic environment increase and organisms can uptake them by ingestion or through the skin. Therefore, the increasing bioconcentration of these substances in the tissue of organisms leads to successively higher levels that move along the food chain. However, this field continues being in an initial phase of development and most of the current data are regarding high concentrations of exposure and the effects of NPs and NMs at low concentrations of exposure in complex mixtures that, to date, have not been well understood (Holden et al., 2016).

The aim of this study is to contribute to the better understanding of toxicity of NMs including AgNPs, AuNPs, $ZrO_2$NPs, $CeO_2$NPs, $TiO_2$NPs, $Al_2O_3$NPs, $C_{60}$ fullerene, graphene, and MPLs (polyethylene and polystyrene) at cell level in terms of oxidative stress and cell viability. The cytotoxic responses of two different cell lines T98G and HeLa, cerebral and epithelial human cells, respectively, that were exposed to the same conditions, were evaluated. Moreover, the synergistic and antagonistic relationships between fullerenes and other organic contaminants, an organophosphate insecticide (malathion), a surfactant (sodium dodecylbenzenesulfonate) and a plasticizer (diethyl phthalate) were assessed. The selection of these three organic compounds was based on their use, their presence and their toxicity in the aquatic environment, as well as their physicochemical properties. Malathion is a commonly used insecticide and in different studies has been related with oxidative stress induction. Sodium dodecylbenzenesulfonate is a commonly detected surfactant in rivers and coastal waters. Furthermore, diethyl phthalate is a widely used plasticizer.

Most of the reported works have explored the cytotoxicity at much higher concentrations in relation to, for example, their applications as drug carriers or nanomedicine. Here, we consider much lower levels, such as those that can be considered by incidental exposure. Notwithstanding, in the case of MPLs almost no data have been reported to date.

## 2. Materials and methods

### 2.1. Chemicals

To the best of our knowledge, the standards and mediums used in this study were of the highest purity available. Dulbecco Modified Eagle's Media (DMEM) and trypsin-EDTA were supplied by Sigma-Aldrich. Fetal Bovine Serum (FBS) and Penicillin/Streptomycin Solution were obtained from Invitrogen™ (Thermo-Fisher Scientific). Dulbecco's phosphate-buffered saline (PBS), dimethyl sulfoxide (DMSO), Hoechst 33258 (bisBenzimide H 33258 ≥ 98%) and dihydroethidium (DHE) (≥ 95%) were purchased from Merck Millipore (Darmstadt, Germany). Salts and analytical standards silver nitrate (ACS reagent, ≥ 99%), trisodium citrate (≥ 98%), gold nanoparticles (10 nm particles size, stabilised suspension in 0.1 mM PBS), zirconium (IV) oxide (suspension 10% wt in $H_2O$, < 100 nm particles size), cerium (IV) oxide (suspension 10% wt in $H_2O$, < 25 nm particles size), titanium (IV) oxide (~21 nm particles size, ≥ 99.5% trace metals basis), fullerene-$C_{60}$, fullerol and fullerene soot (76% of $C_{60}$ fullerene, 22% of $C_{70}$ fullerene and 2% of higher-order fullerenes) produced by the Krätschmer – Huffman method (Kratschmer et al., 1990) was purchased from Sigma-Aldrich (Steinheim, Germany). Aluminium oxide 90 active neutral (0.063–0.200 mm particle size) was purchased from Merck Millipore (Darmstadt, Germany). Graphene nano-powder consisting of 1.5–10 µm particles (4.5 µm mean size) composed of 12 nm graphene flakes (equivalent to 30–50 graphene layers) was supplied by Graphene Supermarket (Reading, MA, USA). The organic compounds used, such as malathion (≥ 99%), diethyl phthalate PESTANAL® (DEHP) (≥ 99.5%) and sodium dodecylbenzenesulfonate ($C_{12}$-LAS) (~80%), were purchased from Sigma-Aldrich.

Silver nanoparticles (AgNPs) were synthetized using trisodium citrate as a reducing agent (Rashid et al., 2013). In brief, all solutions of reacting materials were prepared in HPLC water. Fifty (50) mL of 0.001 M $AgNO_3$ was heated to its boiling point and then 5 mL of 1% trisodium citrate was added, drop by drop. The solutions were heated and stirred vigorously until there was a change of color to pale yellow. Then, the obtained suspension was allowed to reach room temperature. The silver colloid suspension was characterized by nanoparticle tracking analysis (NTA) using a NanoSight LM10 (NanoSight Ltd., Salisbury, UK). This technique allows visualizing particles in liquids, relating Brownian motion and particle size. For this characterization, measurements were carried out in static mode. To adjust camera level and threshold, a pre-scan was carried out.

Polyethylene (PE) microspheres (3–16 µm) accompanied by NPs with sizes between 100 and 600 nm were purchased from Cospheric LLC (Santa Barbara, CA, USA) and polystyrene (PS) (10 µm, 1% solids) accompanied by NP with sizes between 40 and 250 nm were obtained from PolySpherex™ Polystyrene microspheres which are purchased