JOURNAL OF TOXICOLOGY AND ENVIRONMENTAL HEALTH, PART B
2016, VOL. 00, NO. 00, 1–40
http://dx.doi.org/10.1080/10937404.2016.1166466

Taylor & Francis
Taylor & Francis Group

Drosophila Melanogaster as a Suitable In Vivo Model to Determine Potential Side Effects of Nanomaterials: A Review

Mohamed Alaraby[a,b], Balasubramanyam Annangi[a], Ricard Marcos[a,c], and Alba Hernández[a,c]

[a]Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Campus de Bellaterra, Cerdanyola del Vallès, Spain; [b]Zoology Department, Faculty of Sciences, Sohag University, Sohag, Egypt; [c]CIBER Epidemiología y Salud Pública, ISCIII, Madrid, Spain

ABSTRACT

Despite being a relatively new field, nanoscience has been in the forefront among many scientific areas. Nanoparticle materials (NM) present interesting physicochemical characteristics not necessarily found in their bulky forms, and alterations in their size or coating markedly modify their physical, chemical, and biological properties. Due to these novel properties there is a general trend to exploit these NM in several fields of science, particularly in medicine and industry. The increased presence of NM in the environment warrants evaluation of potential harmful effects in order to protect both environment and human exposed populations. Although in vitro approaches are commonly used to determine potential adverse effects of NM, in vivo studies generate data expected to be more relevant for risk assessment. As an in vivo model *Drosophila melanogaster* was previously found to possess reliable utility in determining the biological effects of NM, and thus its usage increased markedly over the last few years. The aims of this review are to present a comprehensive overview of all apparent studies carried out with NM and *Drosophila*, to attain a clear and comprehensive picture of the potential risk of NM exposure to health, and to demonstrate the advantages of using *Drosophila* in nanotoxicological investigations.

Nanotechnology is a new field referring to every technology and science operating on a nanoscale range and to scientific principles and new properties that are found when operating in this range (Siegrist et al. 2007). Nanomaterials (NM) are at the leading edge of the rapidly developing field of nanotechnology (Salata 2004), and nanoparticles (NP) are defined as materials that must be less than 100 nm in at least one dimension. Nanoparticles may include transition metals and metal oxides, silica, carbon compounds (single- and multiwalled carbon nanotubes and fullerenes), nanocrystals, and quantum dots, among others (Dreher 2004; Murray, Kagan, and Bawendi 2000). While bulky materials possess certain properties, regardless of their size, these characteristics of nanoparticulate forms are size and shape dependent. Subsequently, the physical properties, surface-to-volume ratio, and chemical properties of nanoscale materials vary significantly. This is particularly true for metal oxide NP displaying a large surface size, which affects reactivity and other physicochemical properties (Golbamaki et al. 2015). A view of transmission electronic microscopy images of some NP showing different sizes and forms is depicted in Figure 1. As shown, NM are comprised of a broad set of compounds that are difficult to classify. From the risk assessment point of view, NM have been categorized into three different groups according to their physicochemical properties, route of exposure, and mode of action (Gebel et al. 2014). Category 1 includes those NM for which toxicity depends upon properties of its components; category 2 focuses upon fibrous NM; and category 3 includes respirable granular biodurable NP.

Based upon the unique characteristics of NM, nanotechnology is considered to be the next industrial revolution and is postulated to exert enormous impacts on society, economy, and life in general over a period of time (Godwin et al. 2015). In this context, NM have a wide range of applications

CONTACT Ricard Marcos and Alba Hernández ricard.marcos@uab.es; alba.hernandez@uab.es Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Campus de Bellaterra, Cerdanyola del Vallès (Barcelona), Spain.

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/uteb.

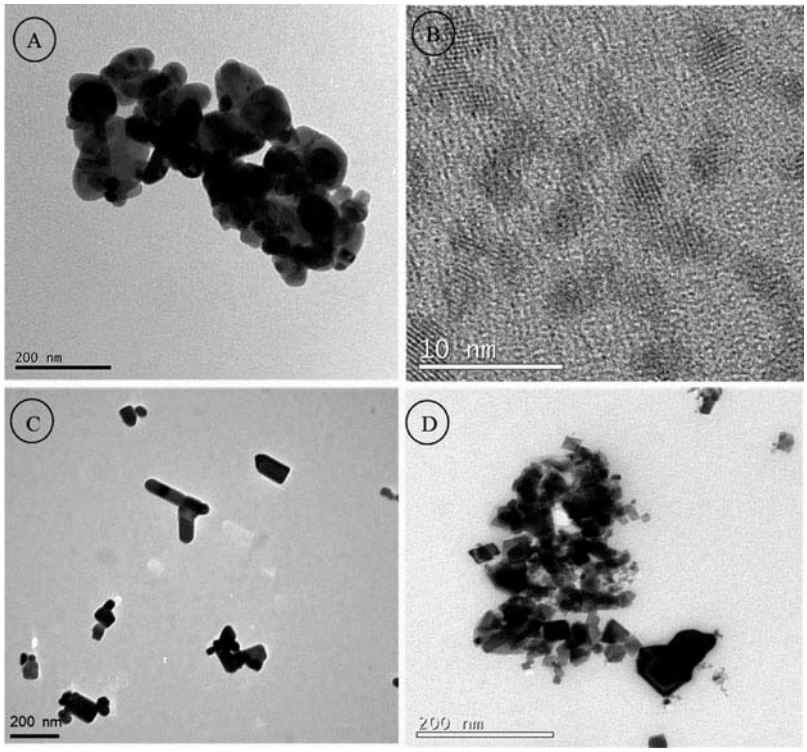


Figure 1. TEM images of different NM: (A) copper oxide nanoparticles; (B) cadmium quantum dots; (C) zinc oxide nanoparticles; and (D) cerium oxide nanoparticles.

ranging from biomedical (used as drug delivery agents, in tumor diagnosis and therapy, in optical imaging, and as antibacterial agents, among others) to electronics, computers, sunscreen products, textiles, paintings, industrial coatings, cosmetics, printer toners, varnishes, and food preservation (Corma et al. 2004; Murthy 2007; Ranjan, Jayakumar, and Zhang 2015; Sato et al. 2004; Snyder-Talkington et al. 2012; Yohan and Chithrani 2014; Zhao and Castranova 2011). The use of NM is expected to increase at an estimated rate of 58,000 tonnes/year during 2011–2020 (Maynard 2006), and nano-enabled products were estimated to generate $731 billion in revenues in 2012 (Lux Research 2014).

The enhanced presence of NM in the environment requires a careful assessment of their unexpected toxicities and biological interactions (Aillon et al. 2009; Chatterjee et al. 2014a; Hawkins et al. 2015; Kermanizadeh et al. 2016; Zhao and Castranova 2011). To reduce the potential interactions of NM within a biological environment, different strategies have been proposed to modify their physicochemical characteristics such as adsorption affinity (Lee et al. 2015). One of these strategies is to change the chemical nature of the polymeric matrix of the NP and thereby alter certain biological phenomena such as biorecognition, biodistribution, bioadhesion, biocompatibility, and biodegradation. A few polymeric materials have been used for this purpose, including gelatin, chitosan, sodium alginate, poly(alkyl cyanoacrylates), poly(lactic acid), poly(lactic-co-glycolic acid), poly[ethylene glycol-co-(lactic-glycolic acid)], poly(ε-caprolactone), and poly(methyl methacrylate) (Couvreur et al. 2002; Liu et al. 2014a; Ma et al. 2013). The monitoring of hazardous effects of NM via in vitro and in vivo models is required to determine their potential harmful effects (Snyder-Talkington et al. 2012). Accordingly, thousands of in vitro and in vivo studies have been conducted to determine the influence and severity following exposure to NM. When testing NM, different factors need to be taken into account to avoid pitfalls, including a good dispersion of the NM to be evaluated (Hartmann et al. 2015). Among the general mechanisms underlying the adverse health effects induced by NM, oxidative stress plays an important role in both toxicity and genotoxicity (Kermanizadeh et al. 2016). Since DNA damage is an initial step in the process of mutations and cancer development, particles that are potent in

producing DNA damage may be regarded as more likely to exert an effect on carcinogenesis (Karlsson et al. 2008). In this context, many investigators showed NM-related cytotoxicity, reactive oxygen species (ROS) production, DNA damage, and genotoxicity (Magdolenova et al. 2014; Oesch and Landsiedel 2012; Willhite et al. 2014).

In vitro studies are relatively simple tests providing a readily available prescreening method using various cell types and culture conditions. In spite of their relevance and importance, these investigations do not completely reflect the actual events that occur in a complete animal with many coordinated tissues and organs (Lewinski, Colvin, and Drezek 2008). The classical in vivo approach using mammalian organisms has important limitations mainly due to the high operational cost and ethical issues. To overcome these problems, more simple in vivo models have been adopted for studying and diagnosis of NM effects. Among these are the roundworm nematode *Caenorhabditis elegans* (Chatterjee et al. 2014a, 2014b; Contreras et al. 2012; Hunt et al. 2013; Meyer and Williams 2014), *Daphnia magna* (Santo et al. 2014; Völker et al. 2013), the zebrafish *Danio rerio* (Dedeh et al. 2015; He, Aker, and Hwang 2014), and *Drosophila melanogaster* (discussed in the following).

Drosophila as a model to evaluate NM harmful effects

The fruit fly *Drosophila melanogaster* is a well-known insect with long scientific history in biological sciences for more than a century and has contributed enormously to understanding the nuances of developmental biology and evolutionary concepts. Further, this dipteran has been used as an ideal model organism to study human diseases including Parkinson's and Alzheimer's, which are regarded as neurodegenerative disorders (Bilen and Bonini 2005), and cardiovascular diseases (Wolf et al. 2006), immunologic and intestinal infections (Kim and Lee 2014), and other human pathologies including cancer (Gonzalez 2013).

The widespread interest of using *Drosophila* as a model and its incorporation into many scientific branches are not simply by chance but may be attributed to the unique characteristics that *Drosophila* possesses, such as a rapid and short life cycle (10–12 days at 25°C, Figure 2), being reliable and cost-efficient (Ong et al. 2015; Vecchio 2015), and the facts that female adults are fertile 12 h posteclosion and a single pair of flies may produce hundreds of offspring within few days (Stocker and Gallant 2008). In addition, *D. melanogaster* has a completely sequenced genome encoding 13,600 genes on 4 chromosomes (Adams et al. 2000). Interestingly, *Drosophila* showed genetic similarity to humans, considering that approximately 75% of the genes involved in human diseases have related or similar sequences to *D. melanogaster* (Lloyd and Taylor 2010). Although *Drosophila* has a simpler nervous system (~200,000 neurons compared to ~100 billion neurons in humans), this species performs complex motor behaviors such as walking, climbing, and flying, and may be trained using fear-conditioning paradigms to test learning and memory (Ambegaokar, Roy, and Jackson 2010). Moreover, the use of *D. melanogaster* is ethically less controversial than using other higher in vivo model organisms (Alaraby et al. 2015c).

The use of *D. melanogaster* in experimental studies has met the standard of the European Centre for the Validation of Alternative Methods (Abolaji et al. 2013) and it is currently employed as a model in toxicology to conduct mechanistic studies on a number of priority environmental contaminants and toxicants (Rand et al. 2014). It is important to point out that the extensive knowledge of the genetics and experimental experiences with *D. melanogaster* have made it unique in terms of usefulness in mutation research and genetic toxicology. *Drosophila melanogaster* has been adopted in mutation research as a specific assay detecting both somatic mutation and recombination as mutant clones induced via loss of heterozygosis (Abdalaziz et al., 2014; Ramel and Magnusson 1992). It should be pointed out that this is the only eukaryote assay that permits quantification of mitotic recombination in somatic cells, which is particularly relevant because aberrant recombination activity is commonly associated with carcinogenesis (Bishop and Schiestl 2003). Further, several investigators successfully employed *D. melanogaster* in the comet assay to detect DNA breaks (Carmona et al. 2011; Marcos and Carmona 2013). All these indicated advantages have prompted many researchers to

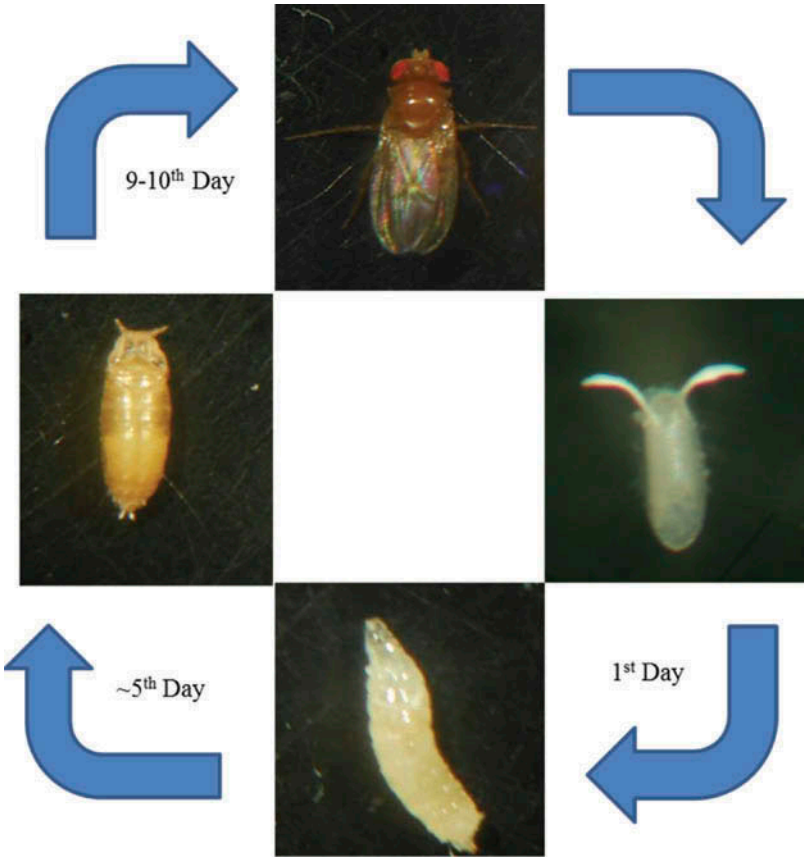


Figure 2. The life cycle of *Drosophila melanogaster* (four stages): egg, larvae, pupae, and adults. After 6–8 h the eggs hatched to give first-instar larvae followed by second and third instars, and at about d 5 the larvae pupate to give the pupa stage. Finally, the adult emerged at d 9 or 10.

use *Drosophila* as model for evaluation of toxicological effects attributed to NM.

Drosophila melanogaster was used for the first time to investigate nanoparticle materials (NM) effects by Strawn, Cohen, and Rzigalinski (2006), to detect exposure effects of cerium NP. At present there are many studies that have used *Drosophila* to test the toxicity, internalization, reactive oxygen species (ROS) production, pigmentation coloring, morphological deformations, gene expression changes, and genotoxicity due to exposure to different NM. In general, it is considered that there are two main routes for NP exposure, that is, via respiration or via food administration, in addition to dermal exposure. Posgai et al. (2009) used *D. melanogaster* as model to assess FluoSpheres, silver NP, and CdSe/ZnS NP of different sizes via inhalation. Several investigators also utilized *Drosophila* as a model organism for oral administration to mimic intake through the intestinal barrier (Alaraby et al. 2015c; Pandey et al. 2013; Siddique et al. 2014). Interest in this in vivo model to evaluate NM is rising, as indicated in Figure 3, and the total number of these studies for different NM is illustrated in Figure 4. A description of the studies conducted with different NM is presented in the following sections.

Studies on silver nanoparticles (AgNP)

The unique optical, electrical, and thermal properties that silver nanoparticles (AgNP) possess place them at the top of all NM used in manufactured materials (Nanotech Project 2014), specifically in relation to their antimicrobial properties (Kim et al. 2007; Li et al. 2010). The nanoparticulate form of Ag is highly cytotoxic and genotoxic at both cellular and whole organism levels, unlike the bulk form of Ag°, which is chemically inert (AshaRani et al. 2008; Dobrzyńska et al. 2014; Gagne et al. 2013; Haberl et al. 2013; Park 2013; Park et al. 2011). Although production of intracellular ROS by AgNP

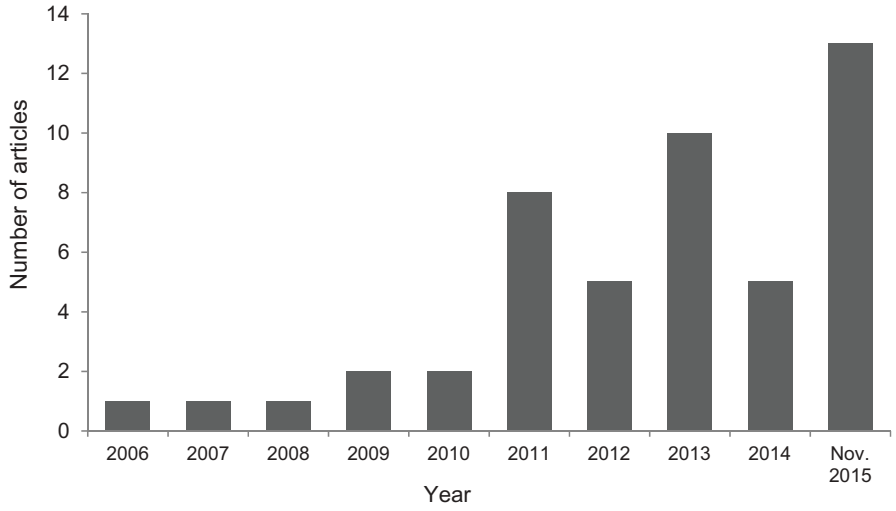


Figure 3. Annual number of published articles that used *Drosophila melanogaster* as in vivo model to evaluate nanomaterials. Collected information covers from the first study (2006) until November 2015, the time of writing this review.

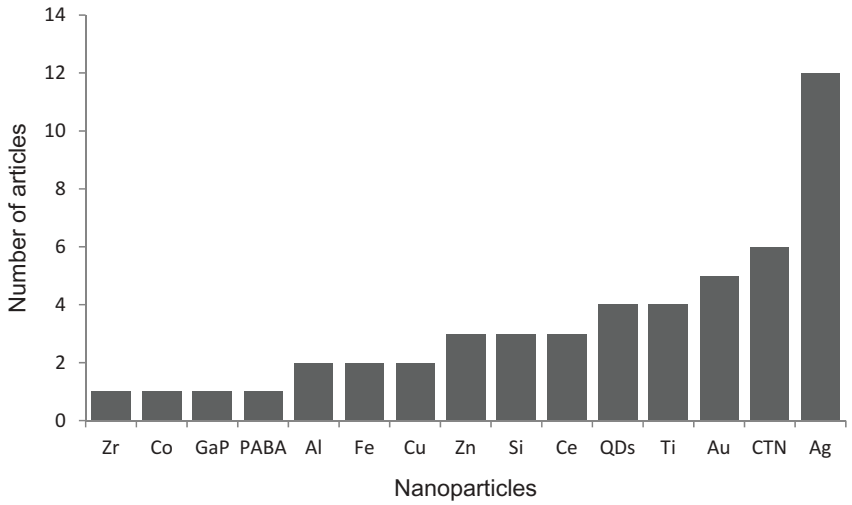


Figure 4. The different types of nanomaterials that have been the subject of studies using *Drosophila melanogaster* as in vivo model for evaluation.

is a well-known mechanism of action, the importance of ion release and endocrine disruption mechanisms is not fully understood (Massarsky, Trudeau, and Moon 2014).

As indicated in Table 1, *Drosophila* has been utilized in several studies aiming to demonstrate the severity of AgNP-induced adverse effects. The first reported study developed a nebulizer-based system to deliver Ag coated and uncoated NP into the respiratory system of adult flies to detect toxicity. The induction of Hsp70 expression in flies was used as a biomarker for toxicity showing significant changes in the expression of both coated and uncoated AgNP in comparison to control. Accordingly, Posgai et al. (2009) proposed the utility of this model to test NM toxicity.

It is known that AgNP induce ROS, as observed in different organisms (Hayashi et al. 2012; Tsyusko et al. 2012). This process may also be modulated by temperature, as observed in the Cape River crab, *Potamonautes perlatus* (Walters et al. 2016). In agreement, oxidative stress pathways including activities of superoxide dismutase (SOD) and catalase (CAT), as well as levels of glutathione (GSH) and malondialdehyde (MDA), are activated in *Drosophila* larvae after AgNP exposure (Ahamed et al. 2010a). Changes in gene expression of SOD and GSH (Armstrong et al.

Table 1. Studies on silver nanoparticles effects using *Drosophila*. Study characteristics.

AgNP (size)	Exposure	Results	Conclusion/recommendations	Reference
Uncoated: 20 nm Polysaccharide coated: 10 nm	Adult stage via respiratory system Dose: 50 µg/ml.	Western blots probed with anti-Hsp70 antibody demonstrated that Hsp70 protein expression was higher in AgNP exposed flies than in controls.	A fundamental merit of this delivery method in toxicity testing confirms the advantage of *Drosophila* as a health model system.	Posgai et al. 2009
Polysaccharide coated: 10 nm	Third-instar larvae exposed to 50 and 100 µg/ml AgNP for 24 and 48 h.	AgNP exposure significantly induced heat-shock stress, oxidative stress, DNA damage, and apoptosis.	The commercial application of Ag NP should be more carefully and thoroughly assessed due to their potential toxic effects to the environment and humans.	Ahamed et al. 2010a
Uncoated: 10 nm Polysaccharide coated: 60 nm	Larvae were exposed to 5–200 µg/ml. 50 m*M* vitamin C used in antioxidant experiments.	Development, mating success and survivorship were affected by exposure. Effects were dose, size, and coating dependent. Each of these effects was partially or fully reversible by vitamin C.	The interaction between coating, size, and agglomeration state in toxicity is clearly complex and needs further study.	Posgai et al. 2011
AgNP: 20 nm AgMPs: 3 µm	Eggs to adulthood were exposed. Dose: 0.005%, 0.05%, 0.5% w/v.	AgNP feeding led to a significant decrease in developmental success, but no decline in fecundity was observed. Observed effects were nanosize dependent.	The unnecessary supplementation of commercial products with AgNP should be restrained at present, until assurance that they will not have detrimental effects on the reproductive health.	Philbrook et al. 2011a
Average size of AgNP: 29 ± 4 nm	Acute toxicity (1 generation): 10 mg/L—100 mg/L Chronic toxicity (8 generations): 5 mg/L.	The acute toxic effect caused 50% of mortality at 20 mg/L. The long-term exposure influenced the fertility during the first three filial generations. The number of individuals with reduced pigmentation increased with AgNP concentration.	It is necessary to pay a great attention to the manipulation and liquidation of materials containing AgNP, considering their possible chronic toxicity impact in the environment.	Panacek et al. 2011
AgNP <60 nm compared with $AgNO_3$	AgNP/$AgNO_3$ doses: 0.1, 1, 5, and 10 m*M* Exposure; 72-h-old-larvae were exposed until pupation.	A significant increase in the frequency of total wing spots in *D. melanogaster* was observed after AgNP exposure, mainly via the induction of somatic recombination. Negative findings were obtained with $AgNO_3$.	The results confirm the useful role of *Drosophila* when the potential health effects of nanoparticles are evaluated.	Demir et al. 2011
Three different sizes: 20–30, 100, and 500–1200 nm	*Drosophila* eggs were exposed to concentrations ranging from 10 to 100 ppm.	20–30 nm AgNP had less effect on the ability of eggs to develop into pupae and adult than both 100 nm and 500–1200 nm. Exposure to 20–30 nm particles did not exhibit a concentration/dependent effect in contrast with 100 and 500–1200 nm nanoparticles.	Nanoscale silver particles (<100 nm) are less toxic to *Drosophila* eggs than silver particles of conventional (>100 nm) size.	Gorth, Rand, and Webster 2011
AgNP characteristics: radius: 16.7 nm, width: 11.3 nm	The concentrations ranged from 0.05% (89.3 ppm) to 5.0% (8930 ppm). Different developmental stages of *Drosophila* were exposed.	Hatch rates were unaffected. Larval progression was impeded at all dosage of AgNP. A distinctive phenotype was observed among emergent adults (F1 generation), which included reduced body pigmentation accompanied by shortened life span and abnormal climbing behavior.	Future studies on the effect of AgNP in whole organisms should focus on delineating relevant pathways related to dopamine synthesis, stress response, or its combination.	Key et al. 2011
AgNP <100 nm compared with $AgNO_3$	Newly emerged adults exposed to 0.1 or 1 µg/ml of AgNP or $AgNO_3$.	Long-term exposure (10 days) at low dose (0.1 and 1 µg/ml) to AgNP significantly shortened life span, but not Ag ions. The expression of *GAL4* was upregulated in response to ingestion of 0.1 µg/ml AgNP.	*Drosophila* may be a versatile terrestrial invertebrate model for studying the effects of AgNP on human health.	Tian et al. 2013

(*Continued*)

Table 1. (Continued).

AgNP (size)	Exposure	Results	Conclusion/recommendations	Reference
Citrate stabilized AgNP 1–50 nm compared with $AgNO_3$	*Drosophila* exposed to 50 mg/L of both Ag compounds.	No effect on survival but fertility and vertical movement ability were affected by AgNP feeding. Tyrosinase and Cu–Zn superoxide dismutase were decreased after AgNP consumption.	*Drosophila*, as established genetic model system, can be well utilized for further understanding of the biological effects of nanoparticles.	Armstrong et al. 2013
Ag NP: 20 nm AgMPs: 7 µm	Doses were 450 µg/ml for both AgMP and AgNP.	AgNP, but not AgMP, had a dramatic negative impact on the development to pupal and adult stages. AgNP decreased the diversity of midgut microbiota from seven orders observed in controls to a single gram-positive order after exposure.	Observed effects can be due to AgNP interactions with intestinal flora. The microbial community had an important role in the observed toxicity.	Han et al. 2014
AgNP size <90 nm	*Drosophila* stage 15 embryos were exposed by microtransferring with doses ranging from 4.3E-08 to 4.3 ng per embryo.	Developmental effects were observed 24 h after exposure to predicted environmental concentrations (PEC).	The direct microtransfer technique enables application of *Drosophila* for in vivo testing of nanomaterial efficacy in a variety of biomedical applications.	Vega-Alvarez et al. 2014
AgNP size 4.7 and 42 nm.	Larvae were treated with 25, 30, and 50 µg/ml of AgNP 4.7 nm; and 250, 500 and 1000 µg/ml of AgNP 42 nm.	Data showed that AgNP at the applied concentrations did not modify the spontaneous frequencies of mutant spots indicating lack of mutagenic and recombinagenic activity. Both AgNP induced pigmentation defects and reduction in locomotor ability in adult flies.	Further experiments must be carried out to gain a better understanding of the mechanism of action of AgNP to ensure their safe use.	Avalos et al. 2015

2013; Posgai et al. 2011) were observed at 50 μg/ml AgNP exposure, confirming potential mechanism of action of AgNP via oxidative stress. Notably, deregulation of antioxidant SOD and GSH enzymes, and reduced developmental effects, loss of survivorship, and short life span were partially or fully reversible by vitamin C treatment, further suggesting the implications of oxidative stress in the adverse effects induced by AgNP exposure (Posgai et al. 2011).

With respect to developmental and viability alterations associated with AgNP exposure, Philbrook et al. (2011a) found that larval feeding led to a significant decrease in developmental success, but without declining fecundity. In contrast, Panacek et al. (2011) observed that chronic exposures to AgNP reduced fertility of flies during the first three filial generations, in addition to diminished developmental alterations. Moreover, marked negative impact on the successful development to pupal and adult stages was noted in larvae exposed to AgNP-containing food media (Han et al. 2014). The adverse effect of AgNP affected not only larvae or adults, but also embryos, where low doses as 4.30E−04 ng applied by microtransferring produced *Drosophila* embryo mortality (Vega-Alvarez et al. 2014).

In agreement with these findings, exposure also reduced adult life span (Tian et al. 2013). Although this toxic effect was not observed in citrate-coated AgNP, this treatment induced loss of melanin pigments in their cuticles (Armstrong et al. 2013). These differences in toxicity might be attributed to physical or chemical characteristics of AgNP, such as different sizes or different coatings. In this context, although exposures to 20–30 nm AgNP did not exert an adverse effect, exposure to 100 and 500–1200 nm AgNP resulted in a significant reduction in larva and pupa viabilities (Gorth, Rand, and Webster 2011).

Drosophila and its gut microbiota have a relevant relationship where the microbiota play an important role in some fundamental aspects of *Drosophila* physiology such as growth control or mating behavior (Charroux and Royet 2012). Notably, gut microbiota directly come into contact with NP as soon as these particles enter into their host gut. In this context, Han et al. (2014) found that AgNP exposure decreased diversity of midgut microbiota by sevenfold to a single gram-positive order. Thus, reduction of microbiota might explain significant viability loss of both pupal and adult stages and negative developmental fitness of larvae observed in AgNP-treated individuals.

The toxicity of AgNP may reflect alterations in the expression of highly conserved gene *Hsp70* (Ahamed et al. 2010a; Posgai et al. 2009). Oxidative stress and upregulation of *Hsp70* after AgNP treatment indicate potential DNA damage, cellular genotoxicity, and finally apoptosis. Accordingly, there are a significantly higher expression of p53 protein, upregulated p38 protein expression in a time- and concentration-dependent manner, and activation of apoptosis pathway via caspase-3 and caspase-9 activation in organisms exposed to AgNP (Ahamed et al. 2010a). Interestingly, the genotoxic effect of AgNP not only induced primary DNA damage, as observed throughout the expression of some genotoxic markers such as p53, but also produced fixed genotoxic damage as evidenced in the wing-spot assay. In this assay AgNP administration promoted induction of a significant rise in frequency of total mutant wing spots (Demir et al. 2011). These genotoxic effects are not in agreement with the recent report by Avalos et al. (2015), who used a high bioactivation *Drosophila* strain, which may explain the observed differences. A review of AgNP genotoxicity data in different systems concluded that these NP may induce DNA damage, chromosomal abnormality, and mutagenicity at noncytotoxic concentrations (Zhang et al., 2014). Interestingly, evidence demonstrated a lack of in vivo studies data that diminish a more accurate risk assessment.

AgNP exposure has been associated with loss of melanin pigments in adult cuticles and with altered adult locomotor behavior (Avalos et al. 2015; Key et al. 2011). In addition, this loss of pigmentation is concentration dependent (Panacek et al. 2011). Interestingly, this demelanization effect does not seem to be attributed to Ag^{+} but to significantly decreased activity of copper- (Cu-) dependent enzymes, such as tyrosinase and Cu–zinc (Zn) SOD. Even though there is a threshold level of Cu present in tissues, consumption of excess AgNP might produce sequestration of tissue Cu, thus creating a condition that resembles Cu loss or starvation. This phenomenon was reaffirmed by addition of excess Cu into the diet, thereby enabling flies to attain normal pigmentation (Armstrong et al. 2013).

Studies with carbon nanotubes (CNT)

Carbon nanotubes (CNT) are cylinders composed of single-walled (SWCNT) or multiwalled (MWCNT) graphene layers with diameters in the nano range and micrometer length (Iijima 1991). Carbon nanotubes have remarkable physical, chemical, electrical, and mechanical properties, allowing them to possess high tensile strength, ultra-light weight, thermal and chemical stability, and excellent semiconductive electronic characteristics (Dresselhaus, Dresselhaus, and Jorio 2004). Such features are the basis for widespread use of CNT in many industrial applications, including electronics, aerospace, construction, and pharmaceutical and biomedical sciences for drug and gene delivery (Asakura et al. 2010; Bianco, Kostarelos, and Prato 2005; Hirsch 2002; Zhang et al. 2014b). Due to specific shape, small size and length, and the addition of side groups (Liu et al. 2013a), CNT exposure has been associated with a variety of detrimental effects, including enhanced production of ROS, inflammation and a decrease in cell viability, necrosis, and genotoxicity (Dong and Ma 2015; Kermanizadeh et al. 2016; Kim and Yu 2014; Lewinski, Colvin, and Drezek 2008; Liu et al. 2013b; Manna et al. 2005; Pulskamp, Diabaté, and Krug 2007; Sato et al. 2005; Shvedova et al. 2003). In addition to the genotoxic potential of CNT, their role in carcinogenesis has also been the subject of different studies, mainly due to their fiber-like similarities to asbestos (Poland et al. 2008). In vivo rodent models have been used to determine the human risk under exposure inhalation, but further studies are required to refine meaningful risk assessments for humans (Oberdörster et al. 2015).

There are in total seven studies that determined the harmful effects of CNT in *Drosophila* (both SWCNT and MWCNT), although only two studies deal with potential genotoxicity (Table 2). Leeuw et al. (2007) exposed larvae to SWCNT to determine biodistribution in different larval compartments. The lack of effects on larvae viability suggested that the quantity of deposited CNT into different compartments was so low as to not initiate any detectable effects. SWCNT and MWCNT were administered during all developmental stages without detectable effects on egg-to-adult survivorship, despite evidence that NM were taken up and became sequestered in tissues. Nevertheless, an important adherence of SWCNT was noted in adults, leading to impaired locomotor function and some increased frequency of mortality (Leeuw et al. 2007). In contrast, MWCNT adhered weakly and did not reduce locomotor function or survivorship (Liu et al. 2009a). This lack of adverse effects by SWCNT was also found in a study where no apparent alterations in larvae viability and growth occurred (Philbrook et al. 2011b). In humans, CNT exposure is mainly via inhalation, and different physicochemical parameters, including shape, state of agglomeration/aggregation, surface properties, impurities, and density, influence toxicity. Interestingly, in the exposure studies using adult flies, CNT were found in spiracles, affecting oxygen diffusion and altering metabolism (Liu et al. 2009a)

A different exposure approach was also employed where CNT were not included in the food media but injected into *Drosophila* embryos. Under these conditions of exposure MWCNT exerted no observable effects on cell motility, cell communication, tissue and organ formation, phagocytosis, or general viability of developing *Drosophila* embryos (Liu, Campo, and Bossing 2014b). Nevertheless, different results were reported using this exposure approach where both SWCNT and MWCNT produced a significant rise in mortality rate, although SWCNT produced equivalent effects at lower doses, indicating greater effects than for MWCNT (Vega-Alvarez et al. 2014). Evidence indicated that the embryonic stage may be considered as a sensitive stage, suitable to be utilized as a model to assess toxicity. Taken together, these observations in *Drosophila* support the general view that SWCNT tend to be more damaging than MWCNT (Jia et al. 2005).

Regarding genotoxic effects of CNT in *Drosophila*, two studies were carried out using the wing-spot assay for detection of both somatic mutation and recombination. Both investigations reported negative results for MWCNT, supporting the view that under the reported exposure conditions MWCNT were not apparently genotoxic. This was true utilizing strains with high metabolic efficiency (Machado et al. 2013) and employing standard strains (de Andrade et al. 2014).

Table 2. Studies on carbon nanotubes (CNTs) effects using *Drosophila*: Study characteristics.

CNTs (size)	Exposure	Results	Conclusion/recommendations	Reference
SWCNTs: width: 1 nm. length: >100 s nm	*Drosophila* larvae were raised on food containing 10 ppm of disaggregated SWNTs.	Larvae viability and growth were not reduced by CNTs ingestion. The biodistribution of CNTs was detected by using near-IR fluorescence microscopy in brain lobes, imaginal disks, salivary glands, Malphigian tubes, trachea, and fat body.	SWNTs ingested have negligible physiological impact. Only a very small fraction (about 10^{-8} of the ingested nanotubes) became incorporated into the organs of the larvae.	Leeuw et al. 2007
SWCNTs and MWCNTs	The doses were 0, 100, and 1000 µg/g of food medium.	No detectable effect on egg to adult survivorship despite evidence that the nanomaterials are taken up and become sequestered in tissue. SWCNTs were adhered extensively to fly surfaces leading to impaired locomotor function and mortality. MWCNTs adhered weakly and did not reduce locomotor function or survivorship.	Clean unexposed flies were observed to be contaminated with nanoparticles through fly-to-fly contact and grooming behaviors. Such redeposition may bring nanoparticles into contact with humans that would not otherwise be exposed.	Liu et al. 2009a
SWCNTs: width: 1–2 nm, length: 5–30 µm	*Drosophila* adults exposed to different concentrations of CNTs (0.005%, 0.01%, 0.05%, 0.1%, and 0.5%, w/v).	No significant effect in egg viability, fecundity and fertility of adults was observed. No impact on the reproductive organs, the lining of the digestive tract, neural tissues and fat body as assessed by histological assessment of whole sections.	In spite of the negative results further studies evaluating developmental toxicity of CNTs needs to be conducted prior to their increased use, particularly in women of reproductive age.	Philbrook et al. 2011b
MWCNTs: surface area: 298 g/m^2, diameter: 10–40 nm, length: 1–2 µm.	*Drosophila* larvae exposed to concentrations ranging from 50 to 250 µg/ml	No effects on the induction of mutant clones in the wing-spot assay. Results were obtained in both strains with basal levels of the cytochrome P-450 and in larvae of high metabolic bioactivity.	Although CNTs were observed in the midgut, perhaps the amount penetrating imaginal disk was not enough to produce genotoxic effects.	Machado et al. 2013
MWCNTs: ID: 5–15 nm, OD: 50–80 nm, length: 10–20 µm	Injected dose equaling to 5 pg of MWCNTs/embryo.	The presence of intracellular MWCNTs has no observable effect on cell motility, cell communication, tissue and organ formation, phagocytosis or general viability of developing *Drosophila* embryos.	*Drosophila* embryos are an excellent system to study developmental effects because of its easy and inexpensive maintenance, ready availability and the possibility of automating embryo injections.	Liu, Campo, and Bossing 2014b
MWCNTs: width: >10 nm	3 ml of CNTs (64, 160, 400, or 1000 µg/ml) in 0.9 g of mashed potatoes medium. 100 larvae were used for each bottle.	No significant increase in mutation and recombination frequencies in the treated series was detected when compared to the negative control group.	Results demonstrated that CNTs do not cause significant genotoxic effects in the SMART assay.	de Andrade et al. 2014
SWCNTs: OD: 1–2 nm, ID: 0.8–1.6 nm, length: 5–30 µm, MWCNTs: OD <8 nm, IO: 2–5 nm, length: 10–30 µm	SWCNTs: 7.2 E-10–7.2 ng MWCNTs: 1 E-06–10 ng *Drosophila* embryos exposed by microtransferring.	SWCNTs induced *Drosophila* embryo mortality from 7.2 E − 04. MWCNTs induced *Drosophila* embryo mortality from 7.2 E–10.	The direct microtransfer method enables application of *Drosophila* for in vivo testing of nanomaterial efficacy in a variety of biomedical applications.	Vega-Alvarez et al. 2014

Discrepancies in the toxicological effects reported by different investigators might be attributed to various factors. First, obtaining good CNT dispersions is difficult and might account for differences in the observed effects (Hartmann et al. 2015). In addition, CNT usually contain small amounts of impurities (Al, Mg, Na, Mn, and Co traces, among others), accounting for a variable percent total weight in endpoint evaluated (Vales, Rubio, and Marcos 2016).

Studies with gold nanoparticles

Gold NP (AuNP) have attracted scientific and technological interest due to their ease in synthesis, chemical stability, and unique optical properties (Alkilany and Murphy 2010). Gold "noble metal" NP were incorporated into many therapeutic and biodiagnostic applications due to many advantageous physical and optical properties. Nevertheless, little is known regarding their intrinsic intracellular effects in biological environments, and for this reason it is necessary to determine possible hazard effects (Austin et al. 2014; Chin et al. 2011; Giljohann et al. 2010).

A summary of investigations conducted with *Drosophila* evaluating the effects of AuNP is presented in Table 3. Different adverse effects of citrate-capped AuNP were initially studied by Pompa et al. (2011) by whole-organism exposure. In such studies a significant reduction of adult life span and fertility was observed after adult and larvae exposure. In addition, enhanced presence of DNA fragmentation and overexpression of stress proteins occurred significantly in gastrointestinal (GIT) cells. These effects were correlated with internalization of AuNP in ovarian and GIT tissues. Further, Pompa et al. (2011) analyzed different biological and developmental aspects of *Drosophila* and noted significant adverse effects on fecundity. In addition, adverse effects were observed, including DNA damage in hemocytes and presence of apoptotic/necrotic events. Induction of genetic damage was confirmed by using the comet assay. Notably, Pompa et al. (2011) demonstrated that genotoxicity resulting from AuNP exposure was related to elevated ROS production and a significant overexpression of p53. To determine potential germinal effects, approximately 10,000 F_1 individuals, resulting from parental individuals exposed to AuNP for an entire life cycle, were analyzed to examine abnormal phenotypes. Important phenotypical changes such as wing deformations and malformations of the eyes were observed. Interestingly, the F_2 generation also showed additional individuals with severely impaired body parts, despite the fact that they arose from parents that did not exhibit any morphological modifications (Vecchio et al. 2012a). To address the potential role of AuNP size and concentration, the harmful effects of different sizes (5, 15, 40, or 80 nm) and a wide dose range (from 0.11 to 467 μg/g/d) were tested. Data indicated that concentration plays a predominant role in determining adverse effects, while size (surface area) of AuNP did not seem to be a key parameter. The observed effects were life-span and fertility values as well as DNA damage. Interestingly, reverse-transcription quanititative polymerase chain reaction (RT-qPCR) experiments validated concentration-dependent toxicity of AuNP, evidenced by overexpression of stress genes (*hsp70* and *hsp83*), DNA damage (*p53*), and apoptotic (*Dronc*) biomarkers. All these data suggested that AuNP effects were related to ROS induction (Vecchio et al. 2012b). The genotoxic potential of AuNP in different in vitro and in vivo systems—including *Drosophila*—was reviewed recently. Investigators concluded that AuNP may induce DNA damage but it was emphasized that there were few existing studies using in vivo mammalian systems (Hadrup et al., 2015). AuNP have also been used to examine effects on metabolism. Wang et al. (2012a) demonstrated that exposure of *Drosophila* larvae to 0.5 or 2 n*M* AuNP resulted in accumulation in the fat body, which is a key metabolic tissue in *Drosophila* larvae. The observed rise in lipid levels was attained without triggering cellular stress responses. In addition, both fatty acid metabolism and the PI3 K/Akt/mTOR signaling pathway were increased. The interesting part of this study is that it reveals a novel function of AuNP in animal metabolism, suggesting its potential therapeutic applications for metabolic disorders (Wang et al. 2012a).

The effects of AuNP in *Drosophila* embryos were recently evaluated in a cost-effective, tissue-specific

Table 3. Studies on gold nanoparticle effects using *Drosophila*: Study characteristics.

AuNP (size)	Exposure	Results	Conclusion/recommendations	Reference
Citrate coated AuNP: 15 nm	*Drosophila* adults and larvae were used: Toxicity/fertility assays: 1.9, 3.8, 19, 38, 190, 380 pmol/L. Gene expression: 380 pmol/L Genotoxicity: 100 pmol/L.	A significant *in vivo* toxicity was observed, as a strong reduction of their life span and fertility. Presence of DNA fragmentation, as well as a significant overexpression of the stress proteins was observed. Transmission electron microscopy demonstrated the localization of the nanoparticles in tissues of *Drosophila*.	The experimental evidence of high in vivo toxicity of nanoscale materials, opens up important questions in many fields, including nanomedicine, material science, health, drug delivery and risk assessment.	Pompa et al. 2011
AuNP: 15 nm	Larvae were fed with 0.5 or 2 n*M* AuNP.	Significant increased lipid levels were observed. Up to 2 n*M* dietary AuNP did not trigger cellular stress responses in larvae, although higher concentration of AuNP might have a mild effect. Activities of the PI3 K/Akt/mTOR signaling pathway and fatty acids synthesis were increased.	This study revealed a novel function of AuNP influencing animal metabolism and suggests its potential therapeutic applications for metabolic disorders.	Wang et al. 2012a
Citrate coated: AuNP: 15 nm	Only one dose of AuNP was tested: 100 p*M*.	A strong perturbation of the health status of the organisms negatively affecting fertility performance. Dramatic phenotypic effects in subsequent generations demonstrate their capability to induce germinal mutagenic effects. Severe DNA damage to the circulating hemocytes and a significant overexpression of p53 were also observed.	Results indicate the need of increases efforts to produce suitable nanoscale surface engineered/coated NM with no hazardous effects for humans and environment.	Vecchio et al. 2012a
Citrate coated AuNP: 5, 15, 40, and 80 nm.	*Drosophila* (adult and larvae) exposed to doses ranging from 0.114 to 467 µg/g.	AuNP induced significant toxicity (strong reduction of lifespan and fertility performance) as well as DNA fragmentation and significant changes in the expression of genes involved in stress responses, DNA damage and apoptosis. Effects were directly related to the concentration but irrespectively of their size.	Results indicate a significant concentration-dependent but size-independent toxicity of AuNP in *Drosophila*.	Vecchio et al. 2012b
AuNP sized <150 nm	*Drosophila* stage 15 embryos were exposed by micro- transferring with doses ranging from 1E-06 to 10 ng per embryo.	AuNP 0.10 and 10 ng induced in *Drosophila* embryo mortality of 26% and 34%, respectively.	*Drosophila* can be used in a variety of disease models and the direct microtransfer technique can be used to test nanomaterial efficacy in biomedical applications.	Vega-Alvarez et al. 2014

NM toxicity assay using direct microtransfer of NM to embryos (Vega-Alvarez et al. 2014). In this case, the monitoring of different embryonic developmental stages permits one to identify specific stages of mortality in only 48 h at environmentally relevant doses. From all the reported studies, it may be concluded that, in spite of the promising future of AuNP in cancer treatment, exposure may potentially involve detrimental effects, indicating that precautions need to be taken into account during the use of this nanoparticulate element.

Studies with titanium dioxide nanoparticles (TiO_2NP)

Titanium dioxide NP (TiO_2NP) are produced abundantly and used widely because of high stability and anticorrosion and photocatalytic properties (Rajh et al. 2014). This NM is among the top five NP used in consumer products, paints, and pharmaceutical preparations. Nevertheless, several investigations demonstrated that TiO_2NP exposure induced toxicity, oxidative stress, DNA damage, and inflammation (Chen et al. 2009; Chen, Yan, and Li 2014; Demir et al. 2013b; Hussain et al. 2005; Liu et al. 2010; Silva et al. 2013).

The potential harmful effects of TiO_2NP were the subject of different studies using *Drosophila melanogaster* (Table 4). In the first study, *Drosophila* larvae were exposed to 5–200 μg NP/ml medium without exerting any apparent adverse effects on development or survival rate (Posgai et al. 2011). However, significant changes in gene expression levels of SOD activity were observed in exposed larvae. These data were in contrast to those indicating that 50-nm TiO_2NP treatments led to a significant progeny loss in *Drosophila*, related to a decline in female fecundity (Philbrook et al. 2011a). Recently Jovanović, Cvetković, and Mitrović (2015) demonstrated that larvae exposure to TiO_2NP did not markedly affect survival but significantly increased time to pupation. Fecundity was unaffected by the treatment, but expression of CAT and that of SOD-2 were markedly downregulated. TiO_2NP were translocated into the larval body but did not develop into adults during metamorphosis associated with some adult individuals presenting aberrant phenotypes (Jovanović, Cvetković, and Mitrović 2015). Toxic effects were also been reported in a recent study using TiO_2NP (<25 nm) that produced cytotoxic effects on midgut and imaginal disc tissues of larvae (Carmona et al. 2015a). This adverse effect of TiO_2NP in *Drosophila* was also noted by using microinjection during the embryo stage. Using this new methodology, Vega-Alvarez et al. (2014) found that environmentally relevant doses of TiO_2NP produced alterations during normal embryo development that suggest potential risk if exposure occurred during the first stages of development.

The potential genotoxic effects of TiO_2NP were also examined in *Drosophila*. Using TiO_2NP with a small size (2.3 nm), and exposing *Drosophila* larvae during all developmental stages, there were no apparent mutagenic effects observed in the wing-spot assay (Demir et al. 2013a). No significant changes in the frequency of all spots (small single, large single, twin, and total multiple wing hair spots and total spots) were observed, indicating that these NP did not appear to induce genotoxicity in the wing-spot assay of *D. melanogaster*. It is interesting that negative findings were obtained with the microparticulate forms, indicating that the nanoparticulate form did not modify potential genotoxicity of its microparticulate versions. Similar negative results in the wing-spot assay were recently reported by Carmona et al. (2015a). However, when primary DNA damage was examined in hemocytes using the comet assay, significant genotoxic effects were detected. It is important to indicate that these results seem to be associated with specific physicochemical properties of TiO_2NP, since no apparent genotoxic responses were observed with the bulk form (Carmona et al. 2015a). Regarding the potential genotoxic risk of TiO_2NP, in vitro approaches have generated a considerably higher number of positive results than in vivo systems, with induction of ROS ascribed as the main mechanism of genotoxicity. In addition, nearly all tests for measuring mutagenicity were negative, as recently reviewed by Chen, Yan, and Li (2014).

Studies with zinc oxide nanoparticles (ZnONP)

Zinc oxide NP (ZnONP), one of the common engineered NM, are widely used in many products,

Table 4. Studies on titanium nanoparticle effects using *Drosophila*. Study characteristics.

TiO_2NP (size)	Exposure	Results	Conclusion/recommendations	Reference
TiO_2NP: length: 38.22 nm, width: 13.53 nm.	Larvae were exposed to 5–200 µg/ml. 50 mM of vitamin C was used as antioxidant.	No effects on development or survivorship, up to doses of 200 µg/ml. Superoxide dismutase and glutathione markers respond to nanotitanium dioxide induced oxidative stress, and this response is reduced by vitamin C.	Since oxidative stress is not translated to biological effect, NP toxicity needs to be examined at different levels of biological organization.	Posgai et al. 2011
TiO_2NP: 50 nm, TiO_2MP: 1–2 µm	Egg to adulthood were exposed to: 0.005%, 0.05%, and 0.5% w/v.	TiO_2NP lead to a significant progeny loss in *Drosophila*, related to a decline in female fecundity.	The unnecessary supplement of commercial products with TiO_2NP should be restrained, until assurance that they will not have detrimental effects on reproduction.	Philbrook et al. 2011a
Q11 TiO_2NP anatase: 2.3 nm	Third-instar larvae exposed to: 0.1, 1, 5, and 10 m*M*.	No significant increases in the frequency of all types of spots (small single, large single, twin, total mwh, and total spots), indicating that these nanoparticles were not able to induce genotoxic activity in the wing spot. Negative data were also obtained with the microparticulate forms.	The fact that TiO_2NP/ionic forms are not genotoxic means that the nanoparticulated form does not modify the toxicity of this element.	Demir et al. 2013
TiO_2NP: <20 nm	*Drosophila* embryo were exposed by microtransferring doses: from 1E-06 to 10 ng.	The highest TiO_2NP doses (0.10, 5.1 and 10 ng) induced embryo mortality of 22%, 28%, and 32%, respectively.	The direct microtransfer technique enables application of *Drosophila* for in vivo testing of nanomaterials in a variety of biomedical applications.	Vega-Alvarez et al. 2014
TiO_2NP anatase: <25 nm Compared to TiO_2MP: 45 µm	Third-instar larvae exposed to doses: 0.08, 0.40, 0.80, and 1.60 mg/ml.	TiO_2NP induce cytotoxic effects on midgut and imaginal disc tissues of larvae, but do not promote genotoxicity in the wing-spot test of *Drosophila*. TiO_2NP exposure in *Drosophila* cause primary DNA damage (results of comet assay), but no effects were observed with the bulk form.	This study remarks the usefulness of using more than one genetic endpoint in the evaluation of the genotoxic potential of nanomaterials.	Carmona et al. 2015a
TiO_2NP as E171, component of food additive 167 ± 50 nm.	Larvae exposed to 0.002–2 mg/ml of TiO_2 in feeding medium.	TiO_2NP showed little toxicity toward *D. melanogaster* at concentrations relevant to oral exposure of humans. Catalase and superoxide dismutase 2 gene expression was markedly down-regulated by the treatment. Thorax malformations were observed.	It is recommended further testing with specific lines of *Drosophila* reared on TiO_2 medium over a multiple generations to fully understand possible risks to humans.	Jovanović, Cvetković, and Mitrović 2015

such as sunscreen products, textiles, paintings, industrial coatings, and antibacterial agents (Contado 2015; Liu et al. 2009b; Montazer and Maali Amiri 2014). These NP are prone to dissolution, and uncertainty remains whether biological/cellular responses to ZnONP are solely due to the release of Zn^{2+} ions (Buerki-Thurnherr et al. 2013), or whether the NP themselves exert additional adverse effects (Mu et al. 2014), or whether effects can be attributed to ROS generation (Sharma, Anderson, and Dhawan 2012). In addition to the ability to induce ROS, ZnONP also interfere with DNA repair mechanisms (Demir, Creus, and Marcos 2014). Independent of the mechanisms of ZnONP-mediated toxicity, large variability noted between studies with respect to potential biological effects of ZnONP suggests that factors such as size and shape may contribute toward overall nanobiological interactions (Saptarshi, Duschl, and Lopata 2015). In fact, in human monocytes ZnONP induced greater cytotoxicity and inflammation than microsized ZnO (Kannan and Vijayaraghavan 2014). Analysis of biological reactivity might be further complicated by factors such as adsorption of proteins on the NP surface, which may influence bioreactivity.

Effects of ZnONP in *Drosophila* are presented in Table 5. Siddique et al. (2014) used for the first time *D. melanogaster* (transgenic *D. melanogaster* (*hsp70-lacZ*)Bg9) to assess the potential toxicity of graphene zinc oxide nanocomposite (GZNC). In this study a time- and concentration-dependent rise in oxidative stress and in the number of apoptotic cells was found. Exposure to higher doses (0.199 and 3.996 μg/ml) of GZNC was toxic after 24 and 48 h, in contrast to lower amounts (0.033 μg/ml and 0.099), which did not produce any apparent adverse effects at both time intervals. In addition to these toxicity studies, genotoxic effects in the comet assay were also reported. These results are in contrast with a recent study showing no apparent toxicity or oxidative stress, and no significant changes in the frequency of mutant clones or percent tail DNA (Alaraby et al. 2015a). In this study *Drosophila* larvae were exposed to ZnONP (<50 nm) or $ZnCl_2$ (ionic form), and adverse effects were not dependent on internalization of ZnONP through the intestinal barrier. Data indicated that neither ZnONP nor $ZnCl_2$ elevated ROS levels that might explain the non-hazardous effect of Zn regardless of its form. However, some deregulation of stress marker genes was noted, since expression of both *Hsp70* and *p53* genes significantly changed due to ZnONP exposure. The differences in the findings of the two previous studies might be due to differences in the chemical composition of ZnONP, where in the first study Siddique et al. (2014) used graphene Zn and possibly the observed effects might be attributed to graphene more than to ZnONP. However, differences in the strains employed (transgenic vs. wild type) also need to be considered as a potential factor for variability.

It is important to point out that Siddique et al. (2015) postulated that Cu-doped ZnONP were nontoxic at concentrations of 1, 2, 4, and 8 μg/μl in *D. melanogaster*, as no apparent loss in climbing and activity pattern was found. In addition, there was no significant change in the activities of acetylcholinesterase (AChE), caspase 9/3, and glutathione-S-transferase (GST) and in levels of GSH, lipid peroxidation (LPO), and total protein content. Further, brain sections displayed no gross alterations in the structure and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) patterns, which revealed no marked changes in protein expression.

The determination of the mutagenic potential of amorphous ZnO and ZnONP has been the subject of two recent papers. In the first study,Reis Éde et al. (2015) noted that amorphous ZnO and ZnONP were not mutagenic in the wing-spot assay. However, the use of a high dose demonstrated that amorphous ZnO (6.25 mM) and ZnONP (12.50 m*M*) produced a significantly increased number of mutant spots compared to negative control. In addition, the observed rise in mutant spots was generated due to mitotic recombination rather than mutational events (Reis Éde et al. 2015). However, no plausible explanation of the potential role of metabolism was provided to describe these weak effects, and a lack of dose response was noted. No genotoxicity in the wing-spot test was reported after amorphous ZnO and ZnONP treatment (Carmona et al. 2015b). However, when both Zn compounds were tested in comet assay using larvae hemocytes, a

Table 5. Studies on zinc oxide nanoparticle effects using *Drosophila*: Study characteristics.

ZnNP (size)	Exposure	Results	Conclusion/recommendations	Reference
Graphene Zn nanocomposites (GZNC: ~9 nm)	Third instar larvae exposed to 0.033, 0.099, 0.199, and 3.996 µg/ml for 24 and 48 h.	Results show a time- and concentration-dependent increase in oxidative stress and in the apoptotic cells and comet tail length, and decreased in total protein, where exposure to higher doses (0.199 and 3.996 µg/ml) of GZNC was toxic for both 24 h and 48 h, in contrast to lower doses (0.033 µg/ml and 0.099) that didn´t show any toxic effects in both time intervals.	*D. melanogaster* showed that toxicity of GZNC is dose but not time dependent. The full implementation of such nanomaterials in biological applications needs to be investigated more.	Siddique et al. 2014
ZnONP: 50–80 nm Compared to $ZnCl_2$	Egg–adult toxicity: from 0.01 to 10 m*M*. For the remaining experiments: from 0.1, to 10 m*M*.	ZnNP were not toxic or genotoxic (SMART and comet assays), and no oxidative damage was observed in haemocytes. The effects induced by $ZnCl_2$ were similar. It is observable that ZnONP induced significant changes in the expression of Hsp70 and p53 genes.	ZnO-NP are non-harmful, at least at the experiment condition. The results confirm the importance of molecular markers such as Hsp70 and p53 to detect effects of low-toxicity materials.	Alaraby et al. 2015a
Doped ZnNP Cu <28 nm	Adult male exposed to 1, 2, 4, and 8 µg/µl.	The results show no loss in the climbing and activity pattern. Also, no significant changes in the levels of AChE, GSH, GST, LPO, caspase 9/3, and total protein content. The brain sections showed no gross changes in the structure, and no changes in the protein expression.	The results suggest that copper-doped ZnONP are non-toxic at 1, 2, 4, and 8 µg/µl in *D. melanogaster*.	Siddique et al. 2015
ZnONP: 20 nm Compared to amorphous ZnO	Larvae treated with 1.60, 3.12, 6.25, or 12.50 m*M* of both Zn forms.	Marker trans-heterozygous individuals from the high bioactivation cross treated with both Zn compounds displayed a significant increased number of mutant spots. The increase in mutant spots was assumed to be due to mitotic recombination, rather than to mutational events.	Due to the genotoxic/recombinagenic potential of Zn compounds its risk assessment is warranted.	Reis Éde et al. 2015
ZnONP: ≤100 nm Compared to ZnO bulk	Larvae feed with 6, 12, 18, and 24 m*M* of ZnONP or ZnO bulk.	The data show that ZnONP genotoxicity in *Drosophila* can be considered as weak according to the lack of mutagenic and recombinagenic effects, and to the induction of primary DNA damage only at high toxic doses.	The genotoxic risk of ZnONP exposure can be considered as low or weak, especially at low doses.	Carmona et al. 2015b

significant elevation in DNA damage was found with ZnONP, but only at the higher dose level. In addition, the lipid peroxidation assay showed a significant increase in malondialdehyde (MDA) levels for both ZnO forms, but elevated MDA concentrations for ZnONP were higher than for ZnO in bulk, suggesting that the observed DNA strand breaks may be mediated by oxidative stress (Carmona et al. 2015b). Overall data suggest that the potential genotoxicity of ZnONP in *Drosophila* might be considered weak according to the lack of mutagenic and recombinogenic responses and induction of primary DNA damage only at high toxic doses of ZnONP (Carmona et al. 2015b). These negative results are in agreement with those recently reported in a long-term in vitro scenario of exposure (Anangi et al., 2015). In these conditions results differ with respect to those noted after acute exposures. Taking into account that *Drosophila* reflects chronic exposure, the results of Annangi et al. (2015a) support the lack of genotoxicity of ZnONP reported in this review.

Studies with quantum dots (QD)

Quantum dots (QD) are fluorescent semiconductors possessing specific photochemical and photophysical properties, with a promising future in medical imaging for disease diagnosis, biosensors, and electronics (Zhao and Zeng 2015). Quantum dots constitute a unique generation of NM, characterized by small size (1–10 nm), with a specific design comprising an internal core and surrounding it by one or two shell layers (Dabbousi et al. 1997; Ji et al. 2014). Quantum dots may be bound with one or several functional groups, as well as specific targeting ligands (Bruchez et al. 1998).

Health risks associated with QD exposure are due not only to their physical shape, but also to their chemical nature, because these compounds possess one or more high-risk heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), or Zn in their core or shell layers (Valizadeh et al. 2012). Subsequently, contact with these heavy metals or atoms in a biological milieu might possibly pose a potential health risk, which is considered an obstacle before design and application of QD.

As indicated in Table 6, different QD have been studied in *Drosophila*. CdSe/ZnS QD-mediated effects were evaluated in *Drosophila*. Posgai et al. (2009) developed an interesting system for inhalation methods. Using this approach, QD of different sizes were effectively aerosolized, showing that the procedure was able to act with a wide range of NP types and sizes. In these cases, red fluorescent CdSe/ZnS NP were successfully delivered to the fly respiratory system and visualized in the tracheal system using fluorescent microscopy. Unfortunately, no toxicity studies were carried out by Posgai et al. (2009). Data suggested that this method was capable of respiratory delivery, providing important information to explore the advantages of *Drosophila* as a health model organism for inhalation effects.

In order to minimize QD-induced toxicity, different strategies such as the use of biocompatible coatings were employed. The role of coatings, as potential modulators of toxicity, was examined using *D. melanogaster* as a model. Galeone et al. (2012) determined and compared the effectiveness of different surface coatings in reducing leaching of metal ions from CdSe-based QD. In vivo toxicity of CdSe–ZnS QD was assessed with different surface coatings: mercaptoundecanoic acid (QD-MUA), polymer coating with poly-maleic anhydride octadecene (QD-PC), and polymer and polyethylene glycol (PEG) coating (QD-PC–PEG). Surprisingly, none of the three different studied coatings complexes completely prevented survival perturbation induced by CdSe-based QD. It is of interest that the observed decreases in *Drosophila* life span were coating dependent, where reductions reached 58, 73, and 82% for QD-MUA, QD-PC, and QD-PC–PEG, respectively.

It needs to be pointed out that the general toxicity associated with Cd based QD exposure is related to Cd^{2+} release (Su et al. 2009, 2010) or elevated levels of ROS (Clift et al. 2010; Gagné et al. 2008). Galeone et al. (2012) found coating-dependent ROS generation linked to the findings that all QD exhibited a marked time-dependent degradation, with significant release of Cd^{2+} ions, explaining the life-span reduction of QD-treated flies. Evidence indicated that the ineffectiveness of different surface coatings to diminish the side effects of Cd-based QD suggested use of

Table 6. Studies on quantum dots effects using *Drosophila*: Study characteristics.

QDs (size)	Exposure	Results	Conclusion/recommendations	Reference
CdSe/ZnS: 5.7 nm	Adult stage via respiratory system: 50 µg/ml.	The red-fluorescing CdSe/ZnS QDs are clearly visible against the green of the tracheal tissue.	This is a simple and cost-effective nebulizer-based method to deliver nanoparticles to the *Drosophila melanogaster* respiratory system, for the purpose of toxicity testing.	Posgai et al. 2009
CdSe–ZnS QDs: QD-MUA: 15 nm, QD-PC: 23 nm, QDPC–PEG: 25 nm	*Drosophila* (adults or larvae) exposed to 200 p*M* of QDs.	QDs with different coat significantly affect *Drosophila* lifespan. QDs induced increased reactive oxygen species (ROS) levels. QDs induced severe genotoxic effects and increased rate of apoptosis in *Drosophila* haemocytes. The toxic effects are related to the in vivo degradation of QDs with consequent release of Cd^{2+} ions.	The coating of QDs can modulate its bioaccumulation in the organism, partly decreasing their overall toxicity. It seems reasonable to suggest careful limitation of human and environmental exposure to Cd-based QDs.	Galeone et al. 2012
InP/ZnS QDs: 11.3 nm CdSe/ZnS QDs: 13.4 nm	100 and 500 p*M* of QDs.	Overexpression of Hsps (70, 83), p53, and Dredd, and high apoptosis rate in flies treated with CdSe/ZnS QDs. Slight effect (only at 500 p*M* dose) in those exposed to InP/ZnS QDs.	CdSe/ZnS QDs are more toxic than InP/ZnS QDs due to the presence of Cd.	Brunetti et al. 2013
CdSe QDs: 3–35 nm Compared to $CdCl_2$	For toxicity and ROS induction: 0, 1, 5, 25, 100, and 500 µ*M*. In the SMART assay: 5, 25, 100, and 500 µ*M*. For the remaining experiments: 25, 100, and 500 µ*M*.	Cd based QDs are toxic, inducing oxidative stress, deregulation of gene expression, and DNA damage. The higher effects observed with $CdCl_2$ indicate that such effects are related to the presence of Cd^{2+} ions.	The increased use of QDs uses may affect human health. Coats are required to avoid cadmium undesirable effects.	Alaraby et al. 2015b

alternatives of QD based upon Cd-free materials, such as indium phosphide (InP)-based QD, may be more beneficial (Brunetti et al. 2013). In this study, Brunetti et al. (2013) exposed *Drosophila* to 100 and 500 p*M* InP-QDs or Cd-QDs, and different parameters including *Hsp70, Hsp 83, p53*, and *Dredd* genes expression were determined, in addition to necrosis and apoptosis induction. Results showed that the two tested doses of Cd-QD produced changes in expression of all analyzed genes, together with a high apoptotic rate. In contrast, gene expression was not markedly altered and low apoptotic rate at the higher dose was noted after InP-QD treatment. These findings indicated that *D. melanogaster* was a sensitive species to detect subtle effects demonstrating, that InP-QD may serve as a safer alternative to Cd-based QD.

In a recent study *D. melanogaster* was also employed to investigate the harmful effects of CdSe QD related to biodegradation (Alaraby et al. 2015b). The potential release of Cd^{2+} from QD was determined by comparing to a free source of Cd ions ($CdCl_2$). Data showed that CdSe QD penetrated the intestinal barrier of the larvae, thereby reaching the hemolymph, interacting with hemocytes, and inducing significant dose- and time-dependent genotoxic effects. Further, ROS production, QD biodegradation, and significant disturbance in the conserved *Hsp*, antioxidant and *p53* genes were also found. Although adverse effects of CdSeQD were noted, these manifestations were still milder than those produced by $CdCl_2$. All these studies indicated the limitations of QD composition related to toxicity.

In different systems, cell uptake, cytotoxicity, and genotoxicity of QD were shown to be dependent on their surface chemistry. Although genotoxicity was related to oxidative stress, this effect only accounted for part of the cytotoxicity and genotoxicity induced by QD. This suggests the existence of other mechanisms of action (Manshian et al. 2016)

Studies with silica nanoparticles (SiO_2NP)

The nanotechnology of silica NP (SiO_2NP) production offers many advantages because these compounds are relatively inexpensive to produce, chemically inert, and thermally stable and may be tailored to contain porous structures for drug encapsulation and hydrophilic for higher solubility in humans (Balakrishnan et al. 2013). For this reason, SiO_2NP are extensively applied in chemical mechanical polishing and as additives to drugs, cosmetics, printer toners, varnishes, and food (Lin et al. 2006), as well as for gene delivery or gene therapy (Baeza and Vallet-Regí 2015; Bharali et al. 2005).

From the toxicological point of view, it was reported that SiO_2NP exposure may produce some adverse effects, including inflammation, release of ROS leading to apoptosis (McCarthy et al. 2012), and cytotoxicity and genotoxicity (Fruijtier-Pölloth 2012; Kwon, Koedrith, and Seo 2014). In addition, Choi et al. (2011) also noted that SiO_2NP exposure produced primary DNA damage and cytotoxicity but not mutagenicity in cultured mammalian cells. However, these results are contradictory since no marked cytotoxic or genotoxic effects were also found by Barnes et al. (2008) and Uboldi et al. (2012). In addition, no morphological transformation was detected (Uboldi et al. 2012).

Three different studies used *Drosophila* to determine whether there is a potential toxic/genotoxic risk attributed to SiO_2NP treatment (Barandeh et al. 2012; Demir et al. 2015; Pandey et al. 2013) (Table 7). Barandeh et al. (2012) organically modified SiO_2NP (20 nm size) and noted that neuronal cell bodies and axonal projections of *Drosophila* were not markedly affected, as evidenced from absence of rise in aberrant neuronal death rate or of interference with normal neuronal processes. In contrast, when SiO_2NP were administered to larvae, a significant uptake was observed in the midgut cells of exposed *Drosophila*. This transposition occurs via endocytic vesicles and by direct membrane penetration after third-instar larvae were exposed orally to 1, 10, or 100 µg/ml for 12–36 h. Subsequently, SiO_2NP internalization led to a significant elevation in levels of oxidative stress as observed in the midgut cells of exposed *Drosophila* in a concentration- and time-dependent manner. SiO_2NP treatment also significantly increased expression of *hsp70* and *hsp22* genes, accompanied by caspases activation, membrane destabilization, and mitochondrial membrane potential loss (Pandey et al. 2013). The observed differences between these studies might be

Table 7. Studies on silica nanoparticle effects using *Drosophila*: Study characteristics.

SiNP (size)	Exposure	Results	Conclusion/recommendations	Reference
Organic modified silica: 20 nm	Larvae exposed to different doses: 0.2, 0.4, and 1 mg/ml.	Although nanoparticles penetrate into living brains, neuronal cell bodies and axonal projections, feeding has no effect on viability. In the neuronal cell body, nanoparticles are present in the cytoplasm, but not in the nucleus.	Results in *Drosophila* indicate that these novel silica based nanoparticles are biocompatible and non-toxic.	Barandeh et al. 2012
Amorphous SiNP: 20–30 nm.	Third-instar larvae exposed to: 1, 10, 100 µg/ml for 12–36 h.	Significant increase in oxidative stress in midgut cells in a concentration- and time-dependent manner. Significantly increased expression of hsp70, 22 along with caspases activation, membrane destabilization, and mitochondrial membrane potential loss. Transmission electron microscopy (TEM) analysis showed uptake in midgut cells via endocytic vesicles and direct membrane penetration.	Ingested aSiNP show adverse effects on the cells of gastrointestinal (GI) tract of the exposed organism; thus, their industrial use as a food additive may raise concern to human health.	Pandey et al. 2013
Synthetic amorphous silica (SAS): 6, 15, 30, and 55 nm. Compared to: SiO_2	Third-instar larvae exposed to: 0.1, 1, 5, and 10 m*M* of the different compounds.	No significant increases in the frequencies of mutant spots were observed in the wing-spot assay with any of the tested compounds. Significant dose-dependent increases in the levels of primary DNA damage, measured by the comet assay, were observed for all the SAS evaluated but mainly when high doses (5 and 10 m*M*) were used. SiO_2 exhibits less toxicity and no genotoxicity.	SAS can induce primary DNA damage, but only when high doses are used. As an overall results show the relevance of using *Drosophila* for testing the genotoxic potential of nanomaterials.	Demir et al. 2015

attributed to different types of SiO_2NP used and also to varying experimental exposure approaches. To assess the potential role of SiO_2NP size in potential toxic/genotoxic-mediated effects, Demir et al. (2015) utilized four different sizes (6, 15, 30, and 55 nm) of the compound. The main goal of this study was to demonstrate the potential induction of somatic mutation and recombination in the wing-spot assay. Thus, when third-instar *Drosophila* larvae were exposed to 0.1, 1, 5, or 10 m*M* of all different sizes of SiO_2NP no significant alterations in frequency of mutant clones were observed. However, all tested SiO_2NP produced a significant dose-dependent elevation in levels of primary DNA damage, measured by comet assay, predominantly with high doses (5 and 10 m*M*). When the comet assay was complemented with the use of formamidopyrimidine DNA glycosylase (FPG) and endonuclease III (EndoIII) enzymes, Demir et al. (2015) found that most of this primary DNA damage corresponded to oxidative DNA damage. Since the higher levels of DNA damage were noted when 55-nm SiO_2NP were utilized, this study indicates the importance of size when testing the biological effects of NM and the need to include different sized compounds.

A recent revision of in vitro and in vivo studies carried out with SiO_2NP produced inconclusive results on their potential genotoxic risk due to a variety of factors. Data suggested that although SiO_2NP might interact with outer and inner membranes, signaling responses, and vesicle trafficking pathways, the potential mechanisms of toxicity as well as the implication in human health have not been fully elucidated (Kwon, Koedrith, and Seo 2014).

Studies with cerium nanoparticles (CeO_2NP)

Cerium oxide NP (CeO_2NP), as with other NM, are extensively used in many applications, including catalysts, solar cells, solid fuel cells, ultraviolet absorbents, automotive catalytic converters, gas sensors, oxygen pumps, and metallurgical and glass/ceramic applications (Corma et al. 2004; Izu et al. 2004; Walkey et al. 2015). At present the importance of CeO_2NP is attributed to their antioxidant properties such as scavenging ROS, together with an ability to inhibit cardiovascular myopathies and to protect normal cells from radiation effects (Wason and Zhao 2013). This provides CeO_2NP a promising future in pharmacology (Celardo et al. 2011) and medicine (Das et al. 2013). Despite the positive characteristics of CeO_2NP, several studies reported some adverse effects including enhanced ROS generation, cytotoxicity, genotoxicity, and inflammation (Ali et al. 2015; Eom and Choi 2009; Thill et al. 2006).

Until now, only three studies have utilized *Drosophila*, as presented in Table 8. Interestingly, all the studies reported positive effects on the health status of *Drosophila* with increasing life span (Cohen et al. 2008; Strawn, Cohen, and Rzigalinski 2006) or showing antitoxic and antigenotoxic effects (Alaraby et al. 2015c). Strawn, Cohen, and Rzigalinski (2006) found that administration of CeO_2NP extended fly life span up to 38% and increased survival after a lethal exposure to paraquat. In addition, CeO_2NP elevated median life span in females by 18 d, and maximum life span by 19 d, with an overall rise in activity in aged females (Cohen et al. 2008). These results in *Drosophila* are supported by the findings of Alaraby et al. (2015c), who demonstrated no significant toxic effects following CeO_2NP or Ce-sulfate (ionic form of cerium) administration at doses as high as 10 m*M* in the culture medium. There were no apparent malformations observed in adults upon metamorphosis of larvae that were grown in exposed medium, but a significant reduction in frequency of spontaneous duplications of scutellar bristles was noted. Similarly, the potential genotoxic effects of CeO_2NP were evaluated and data showed that CeO_2NP did not induce somatic mutations or recombination in the wing imaginal discs of treated larvae. Moreover, *Drosophila* larvae pretreated with CeO_2NP (0.1, 1, or 10 m*M*) significantly reduced the severity of genetic damage induced by potassium chromate (0.5 m*M*) post exposure, compared to potassium chromate treatment alone. It is worthwhile noting that the antigenotoxic effects observed for CeO_2NP were related to the antioxidant properties of CeO_2NP, especially at the higher doses tested, where low levels of ROS were generated. The antigenotoxic and antioxidant properties of CeO_2NP did not completely prevent potential side effects. Thus, a deregulation in the expression of *Hsps* (70, 26, 60, and 83) genes was found at the highest dose used (10 m*M*), and some abnormal-shape mitochondria

Table 8. Studies on cerium nanoparticle effects using *Drosophila*: Study characteristics.

CeNP (size)	Exposure	Results	Conclusion/recommendations	Reference
CeO_2NP (no size indicated)	CeO_2NP doses: from 0 to 100 µ*M* Larvae and adults were used.	In females, treatment with CeONP increased median life span and maximum life span, as well as overall activity.	Results suggest that CeONP may provide effective future treatment for free-radical-mediated disorders, opening a new realm of pharmaceutical development.	Cohen et al. 2008
CeO_2NP (no size indicated)	CeO_2NP doses: from 0 to 100 µ*M*. Larvae and adults were used.	In *Drosophila*, administration of CeO_2NP extended fly life span up to 38% and induced survival after a lethal exposure to paraquat by 200%.	Results suggest that nanotechnology may be used in a new realm of nano-pharmacology, with novel alternatives for treatment of disease states.	Strawn, Cohen, and Rzigalinski 2006
CeO_2NP: < 25 nm Compared with: cerium sulphate	Toxicity experiment: 0.01, 0.1, 0.5, 1, 5, and 10 m*M*. Genotoxicity (SMART assay): 72-h larvae exposed to 0.1, 1, and 10 m*M*).	TEM images showed internalization of Ce-NP throughout the intestinal barrier reaching to haemocytes. Neither toxicity nor genotoxicity related to both forms of cerium was observed. CeO_2NP significantly reduced the genotoxic effect of potassium dichromate and the intracellular ROS production.	The study highlights the importance role of *Drosophila* intestinal barrier in the transposition of nanomaterials entering via ingestion. The results indicate the antioxidant and antigenotoxic properties of cerium NP.	Alaraby et al. 2015c

were observed in the cells of the larval midgut. These results demonstrated that CeO_2NP possess unique advantageous properties but also exert some negative effects.

Despite the reported negative genotoxic effects in different studies, an important number of experiments obtained negative results associated with antigenotoxic potential as reported by Alaraby et al. (2015c) in *Drosophila*. In human cells, the antioxidant and antigenotoxic effect of CeO_2NP has been recently reported (Rubio et al. 2016)

Studies with copper nanoparticles (CuNP)

The increased presence of copper NP (CuNP) in our immediate environment due to widespread usage in electronics, textiles, and electronic devices, as well as with antimicrobial agents, raises a question regarding potential adverse risk to humans (Rubilar et al. 2013). Karlsson et al. (2008) reported that copper oxide NP (CuONP) and Cu-based compounds are more toxic than other metal oxides. In this context, CuONP were found to induce adverse and genotoxic effects at cellular level in studies in vitro (Ahamed et al. 2010b; Akhtar et al. 2013; Jing et al. 2015; Wang et al. 2012b), as well as in whole-animal studies (Adam et al. 2015; Gomes et al. 2013; Isani et al. 2013; Lei et al. 2008; Liao and Liu 2012; Nations et al. 2015; Wang et al. 2015). Similar to other NP, there is a debate around the main causative factor underlying CuONP-mediated adverse effects, whether attributed to CuNP or leaching of soluble ions from these compounds. However, there are still insufficient data and no agreement has been reached (Park et al. 2014) regarding mechanisms underlying CuONP-induced toxicity. Four studies were conducted using *D. melanogaster* as a model organism to determine potential harmful effects attributed to CuONP (Alaraby, Hernández, and Marcos 2015d; Carmona et al. 2015c; Han et al. 2014; Siddique et al. 2013) (Table 9).

Although CuONP possess the ability to penetrate midgut cells and subsequently translocate to the general body compartment (internal hemolymph), the abilities to (1) reduce larval growth, (2) decrease flies viability, and (3) delay emergency periods were only detected at higher doses (2 and 10 m*M*) during larval development (Alaraby, Hernández, and Marcos 2015d). Similar results were previously reported when *D. melanogaster* was exposed to low doses (0.033 μg/ml) graphene copper nanocomposite (GCNC). At this dose no marked toxic effects were found at exposures lasting for 24 and 48 h when several parameters including hsp70 expression, β-galactosidase activity, oxidative stress, total protein, and apoptotic rate and DNA damage were examined; however, at higher doses of GCNC (0.199 and 3.996 μg/ml) the adverse effects occurred at both 24 and 48 h (Siddique et al. 2013). In contrast, CuNP exposure did not appear to exert adverse effects since no developmental-stages manifestations were observed (Han et al. 2014). This study only showed significant increases in the time spent for adult transition without negative impact on larval fitness.

On the potential genotoxic effects of CuONP, Carmona et al. (2015c) demonstrated that treatment induced DNA strand breaks, accompanied by elevated frequency of somatic mutations and recombination events, which might partially explain oxidative stress generation. The observed genotoxic effects were close to the threshold level. This marginality of these reported values might explain the lack of effects noted in the study of Alaraby, Hernández, and Marcos (2015d), where CuONP failed to demonstrate genotoxic potential, possibly due to the inability to enhance ROS production. This apparent lack of genotoxic effect did not prevent the influence on deregulation in expression of different stress genes, including antioxidant-related ones. Interestingly, CuONP exposure significantly reduced expression of different genes (*Duox, Upd3, PPO2*, and *Hml*) involved in intestinal barrier integrity. These genes were proposed as suitable markers to determine potential damage induced by NP in the intestinal barrier (Alaraby, Hernández, and Marcos 2015d). With regard to the potential role of Cu ions in CuONP exposure effects, results indicated that effects produced by $CuSO_4$ are an important source of Cu ions that were always higher than those found with CuONP. These data support the view that the nanoparticulate form is responsible for most of the observed harmful effects.

In general, studies on the genotoxicity of CuONP by using approaches other than

Table 9. Studies on copper NP using *Drosophila*: Study characteristics.

NP (size)	Exposure	Results	Conclusion/recommendations	Reference
Graphene copper nanocomposite				
GCNC: ~4 nm	Third-instar transgenic (hsp70-lacZ)Bg^{9} larvae exposed to 0.033, 0.099, 0.199 and 3.996 µg/ml for 24 and 48 h.	Toxicity was determined throughout hsp70 expression, β-galactosidase activity, oxidative stress, total protein, and apoptotic rate and DNA damage. Results showed that the exposure to GCNC were toxic increasing all the toxicity markers used.	GCNC is toxic especially at higher concentration tested. The full implementation of such nanomaterials needs to be more investigated.	Siddique et al. 2013
Copper NP				
CuONP: 20 nm compared with CuOMPs: 1.5 µm	The dose of 50 µg/ml was used for egg survival, and 500 µg/ml for adult survival.	Neither CuONP nor CuOMP appears to affect developmental stages, with no negative impact on larval fitness. Nevertheless, exposure increased the time spent in adult transition.	The gut microbial community had an important role in the observed toxicity. Microbiota should be examined to fully understand the impact of nanomaterials	Han et al. 2014
CuONP: <50 nm, compared with $CuSO_4$	Third instar larvae exposed to 0.24, 0.48, and 0.95 mg/ml of CuONP and 0.25, 0.50, and 0.75 mg/ml or $CuSO_4$.	CuONP can induce DNA strand breaks, and increase somatic mutation and recombination frequency. These effects can be explained partially by oxidative stress.	This study supports the value of *Drosophila melanogaster* as a model organism in nanogenotoxic studies.	Carmona et al. 2015c
CuONP: <50 nm, compared with $CuSO_4$	A wide range of doses used: 0.016, 0.08, 0.4, 2, 5, and 10 m*M*, for both compounds. Exposure takes place during larval development.	CuONP have the ability to internalize through *Drosophila* tissues inducing deregulation in the expression of genes related to physical interaction of NP with the intestinal barrier leading to toxicity at high exposure doses. No ROS induction or genotoxic effects were observed. The effects induced by $CuSO_4$ in the different targets evaluated were always higher than those observed after CuONP exposure.	The results indicate that the detrimental effects observed are mainly due to the release of Cu^{2+} ions more than to the NP form. New genes (*Duox, Upd3, PPO2*, and *Hml)* are good biomarkers to determine injury on the intestinal barrier.	Alaraby, Hernández, and Marcos 2015d

Drosophila are relatively scarce. A summary of the published data indicates that changes in gene expression, apoptosis, oxidative stress, and persistent inflammation were noted (Magaye et al. 2012). Nevertheless, the number of in vivo studies performed is far lower than in vitro investigations.

Studies using other nanoparticles

In addition to the studies reported in the preceding, there are eight more studies that employed *D. melanogaster* to determine potential effects of aluminum NP (Demir et al. 2013a; Huang et al. 2013), cobalt NP (Vales et al. 2013), zirconium NP (Demir et al. 2013a), iron NP (Chen et al. 2015; Vega-Alvarez et al. 2014), gallium nanowires (Adolfsson et al. 2013), and PABA (4-*N*-pyridin-2-yl-benzamide nanotubes) (Yadav et al. 2010), as presented in Table 10.

Aluminum (Al)-based NP are widely used in various fields, including manufacturing, food, cosmetics, and medicines. Although Al NP were suggested to play a role as a potential neurotoxin, there is no general agreement among researchers on health risk posed (Willhite et al. 2014). Huang et al. (2013) observed that in isolated brains of *Drosophila* after 15 min of application of alumina NP (2 mmol/L), the average frequencies of spontaneous rhythmic activities in the antennal lobe of *Drosophila* were significantly decreased compared with controls, indicating a neurotoxic manifestation. Toxicity and genotoxicity studies carried out with aluminum oxide NP (Al_2O_3NP) noted a low toxic and genotoxic potential compared with other metal NP (Rajiv et al. 2016). This is in agreement with the results obtained in *Drosophila* when genotoxicity was evaluated by Demir et al. (2013a), who found no significant alterations in frequency of mutant spots in the wing-spot assay, indicating that these NP did not induce genotoxic (somatic mutation and/or recombination) responses in *D. melanogaster*. Interestingly, negative results were also detected in the same study when a micrometer-sized Al_2O_3 form was tested.

Cobalt-based NP (CoNP) are used in medicine as a highly effective magnetic resonance imaging contrast agent, as well as a chrome–cobalt alloys in many implants. Although there is a lack of well-designed toxicity, genotoxicity, and carcinogenicity studies, Magaye et al. (2012) demonstrated that changes in gene expression, apoptosis, oxidative stress, and persistent inflammation were major consequences associated with CoNP exposure, which may predispose to carcinogenicity. In fact, in mammalian cells CoNP was shown to induce cell transformation after long-term (12 wk) exposure to subtoxic concentrations, and these effects were mediated by oxidative stress (Annangi et al. 2015b). Vales et al. (2013) found in 72-h-old *Drosophila* larvae exposed to a wide range of doses (0.1 to 10 m*M*) of CoNP that this failed to markedly affect the frequency of mutant clones. Interestingly, the genotoxic effects of CoNP were higher than those produced by the ionic form (cobalt chloride), suggesting that Co ions are not a major health risk associated with CoNP exposure.

Zirconium (Zr), at both nano- and microscale, is widely used in applications like biosensors, cancer therapy, and implants. It was postulated that ZrNP are not apparently toxic but that these NP possess effective antioxidant potential. The antioxidant effects are more marked than those observed with microparticulate forms (Karunakaran et al. 2013). With regard to genotoxic risk, although there are no studies on ZrNP, the existing in vitro studies conducted with ZrO_2 demonstrated that nongenetic damage was reported in peripheral blood lymphocytes and murine fibroblasts when comet assay was used (Braz et al. 2008; Ribeiro et al. 2009). This lack of genotoxicity is in agreement with the only study that was carried out in *Drosophila* (Demir et al. 2013a). Data obtained in this study demonstrated that no significant alterations in frequency of mutant spots occurred in the wing-spot assay, indicating that neither ZrNP nor its microparticulate forms induced genotoxic responses in the wing-spot assay of *D. melanogaster*.

Iron oxide (FeO) NP are considered as biocompatible, biodegradable, and nontoxic and for these reasons are utilized for a wide range of biomedical applications such as tumors or vascular imaging, drug delivery, gene therapy, and as an iron supplement for patients with anemia. Based upon various investigations conducted to determine potential toxic/genotoxic effects, it seemed that characteristics such as core size, morphology, surface charge, and type of coating were important to modulate these effects (Arami et al. 2015). Two studies using

Table 10. Studies on other NP using *Drosophila*. Study characteristics.

NP (size)	Exposure	Results	Conclusion/recommendations	Reference
Aluminium NP				
Alumina-NP: 18.9 nm Compared to submicrometer alumina 402.1 nm	Dose was 2.0 m*M* to isolated brains.	Fifteen min after exposure the average frequencies of rhythmic spontaneous activities of the antennal lobe were significantly decreased compared with control groups.	These results indicated that nano-alumina might have adverse effects on the central nervous system in *Drosophila*.	Huang et al. 2013
Al_2O_3NPS: 16.7 nm Compared to Al_2O_3	Third-instar larvae were exposed to 0.1, 1, 5, and 10 m*M*.	Results showed no significant increases in the frequency of mutant spots, indicating that these nanoparticles were not able to induce genotoxic activity in the wing-spot assay of *D. melanogaster*. Negative data were also obtained with the microparticulate Al_2O_3 form.	Aluminium in both NP/ionic forms was unable to produce genotoxic effects in *Drosophila*, indicating that the NP form does not modify the potential genotoxicity of their ionic form.	Demir et al. 2013
Cobalt NP				
CoNP: <50 nm Compared to cobalt chloride	72-h-larvae exposed to 0.1, 1, 5, and 10 m*M*. Larvae were fed on this treated medium until pupation.	The results indicate that both cobalt forms induce significant increases in the frequency of mutant clones. The genotoxic effects induced by CoNP in the wing-spot assay are due to somatic recombination.	CoNP showed higher genotoxic potential than cobalt chloride in an in vivo model such as *Drosophila*.	Vales et al. 2013
Zirconium NP				
ZrO_2NP: 6 nm Compared to ZrO_2	Third-instar larvae were exposed to 0.1, 1, 5, and 10 m*M*.	No significant increases in the frequency of any type of mutant spots were observed indicating that these NP were unable to induce genotoxic activity in the wing-spot assay of *D. melanogaster*. No effects were also obtained with the ionic form.	Zirconium independently of its form was unable to produce genotoxic effects in *Drosophila*.	Demir et al., 2013
Iron NP				
Fe_3O_4NP: 12 nm Coated with carboxy methyl dextran	*Drosophila* embryo were exposed by microtransferring doses: from 6.5E-08 to 5 ng.	The highest doses (2.5 and 10 ng) induced embryo mortality of 328 and 46%, respectively.	The direct microtransfer technique enables application of *Drosophila* for in vivo testing of nanomaterials in a variety of biomedical applications.	Vega-Alvarez et al. 2014
Magnetite NP: UN-MNP (neutral), CA-MNP (negative), APTS-MNP (positive). Size for all: 15 nm.	MNP were added to *Drosophila* growth medium at doses of 0, 300, and 600 µg/g.	The uptake of MNP caused reduction in the female fecundity, and developmental delay at the egg–pupae and pupae–adult transitions. The parental uptake disturbs the oogenesis period, induces ovarian defect, reduces the length of eggs, decreases the number of nurse cells, and delays egg chamber development. The dyshomeostasis of Fe along the anterior–posterior axis of the fertilized eggs was associated with development.	The surface modification of MNP shows different effects on egg development. Comparatively, the positively charged surface induced more developmental defects than neutral and negative surface charged.	Chen et al. 2015
Gallium phosphide nanowires GaP				
Size: 80 nm	Larvae: Exposed to 10 nanowires nl^{-1}. Adults exposed to 50 µl of 6×10^7 nanowires ml^{-1} water.	Results show that GaP nanowires applied through the diet are not taken up into *Drosophila* tissues, do not elicit a measurable immune response or changes in genome-wide gene expression and do not significantly affect life span or somatic mutation rate.	Results suggest that the *Drosophila* intestinal tract is relatively insensitive to nanowire exposure and protects the adjoining tissues from environmental hazards.	Adolfsson et al. 2013

(*Continued*)

Table 10. (Continued).

NP (size)	Exposure	Results	Conclusion/recommendations	Reference
PABA: 4-*N*-pyridin-2-yl-benzamide nanotubes				
Size: 3–5 nm To compounds (PNT-A and PNT-B) with different fluorescence	Larvae were exposed to 60 µg/100 µl in the growth medium.	A distinct distribution of the two separate nanotubes was observed in various internal organs of *Drosophila*. Exposure of PABA nanotubes does not produce any hazards, including locomotion defects and mortality of adult flies.	The use of self-assembled nanotubes can add new dimensions and scope to the development of dual-purpose oral carriers for the fulfilment of many biological promises.	Yadav et al. 2010

Drosophila were carried out to examine the influence of FeO NP (Chen et al. 2015; Vega-Alvarez et al. 2014). Vega-Alvarez et al. (2014) aimed to detect adverse effects in the embryo by microinjecting NP directly into the embryo. Using this approach, FeO NP at doses of 2.5 and 5 ng induced significant increases in rate of mortality, indicating that these NP are toxic if they reach the embryo during the first stages of development. Chen et al. (2015) showed that relatively high doses (300 and 600 μg/g in food media) of magnetite NP affected general survival and fecundity in young virgin females. Evidence indicated that Fe magnetite NP penetrated *Drosophila* placental tissue and entered into the ovoplasm and vitelline membrane of the eggs, producing reduction in female fecundity and developmental delay at the egg–pupae and pupae–adult transitions. The reduction in fertility is the consequence of induced defects in ovaries, reduction in the size of egg chamber, and number of nurse cells in egg chambers. These effects were more severe when the positive 3-aminopropyltriethoxylsilane coated iron magnetite NP were used.

Gallium phosphide (GaP) nanowires are another type of NP that were tested in *D. melanogaster* (Adolfsson et al. 2013). Data obtained as results demonstrated that GaP nanowires introduced into larvae and/or adults food were not taken up into *Drosophila* tissues, did not elicit a measurable immune response or changes in genome-wide gene expression, and did not significantly affect life span or somatic mutation rate. These results indicated that *Drosophila* intestinal tract was relatively insensitive to nanowire exposure and protected the adjoining tissues effectively from environmental hazards.

PABA (*p*-aminobenozic acid) is a natural nonprotein amino acid that is frequently found as a structural moiety in drugs with a wide range of therapeutic application. The design and chemical synthesis of PABA derivatives and its self-assembly to form orally ingested fluorescent PABA nanotubes have been evaluated in *Drosophila* (Yadav et al. 2010). The bioavailability of two different nanostructures and distribution in various internal organs of *Drosophila* were examined. The observed results indicated that bioaccumulation of NM in different organs was dependent upon the side-chain modifications. In addition, neither of the PABA nanotubes produced significant health hazards, including locomotion defects and mortality of adult flies. The main conclusion of this study is that despite differential uptake and clearance from multiple tissues, the use of self-assembled nanotubes may add new dimensions and scope to the development of drug oral carriers.

Discussion and general conclusions

From all the studies reviewed, it is clear that *Drosophila melanogaster* may be considered a qualified in vivo model that succeeds in assessing NM effects. As initially indicated, among the advantages of using *Drosophila* there are the simplicity in handling, low cost of maintenance and use of *Drosophila* strains, and, mainly, the important amount of information that exists on this organism as the result of many years of experiences in many biological fields. As a negative factor one needs to be aware of the phylogenetic differences that exist between flies and humans in terms of interpolation of effects. This creates a risk to extrapolating *Drosophila* data to humans. Nevertheless, there are many studies linking *Drosophila* use as a model for different human pathologies (Foriel et al. 2015; Maximino et al. 2015; Plantié et al. 2015; Venken et al. 2016). As an in vivo model the influence of NM may be evaluated at different levels, from overall toxicity to genotoxicity. In addition, effects on physiology, behavior, and locomotive effects, among others, can also be assessed. *Drosophila* shows a high sensitivity to changes not only in chemical composition of NM but also of different coating or physical variations in size, shape, and surface area. Moreover, *Drosophila* may be used as an effective sensitive biomonitor to determine shedding of ions from NM and subsequent physical interactions. The potential release of ions is an important point that requires being considered. NM might release ions depending on the surrounding matrix (Handy et al. 2012). *Drosophila* exposure to NM usually occurs via food medium, a complex matrix where different interactions occur, including the release of ions. To avoid misinterpretations of the obtained data it is suggested to complement the use of NM with their chemical ionic forms (Alaraby et al. 2015a).

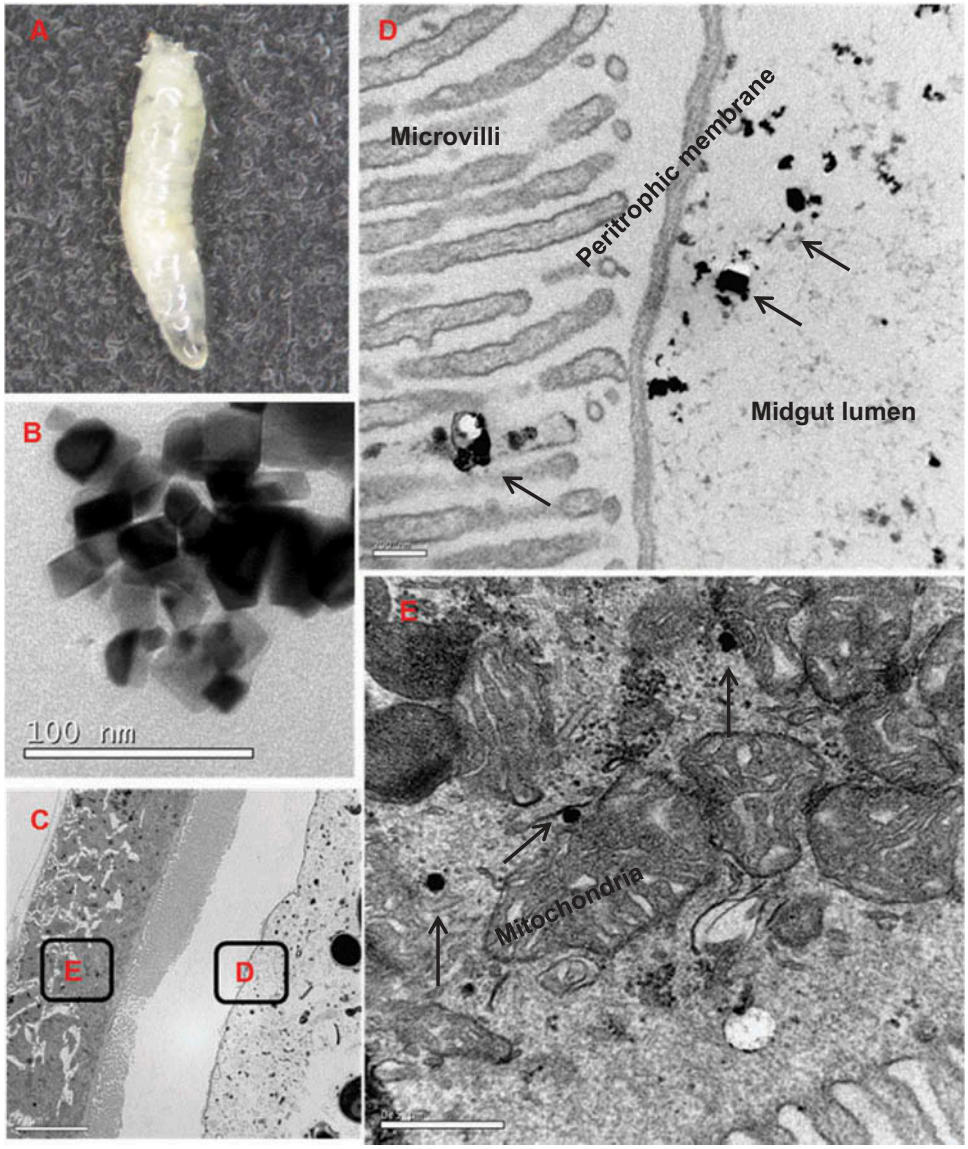
In this way, more accurate comparison between NM and ionic form effects might be obtained. In addition, *Drosophila* offer a reliable model to measure NM effects in all life stages from embryo up to adult, passing through transitional stages in a relative short time. Since most of the studies were carried out by exposing larvae via oral administration, *Drosophila* became a reliable model to evaluate the role of the intestinal barrier against NM. In *Drosophila* the intestinal barrier includes the peritrophic membrane (PM) and one layer composed of a large midgut enterocytes. PM is formed from chitin-containing microfibers embedded in a matrix, and the principal constituents of these are proteins, glycoproteins, and mucopolysaccharides (Spence 1991). PM is considered analogous to the mucus layer of the mammalian digestive tract, and shields the midgut epithelium from abrasive food particles and microbes (Hegedus et al. 2009). PM does not permit entry of particles of the size of most viruses, bacteria, protozoans, and helminthes (Spence 1991). In addition, PM protects the midgut cells from sharp materials and from xenobiotics and toxins, such as dichlorodiphenyltrichloroethane (DDT) and *Bacillus thuringiensis* toxins (Hayakawa et al. 2004; Tellam 1996).

Alaraby et al. (2015c) found that CeNP were distributed inside midgut lumen, attached to PM, and present in midgut cells and hemolymph (Figure 5). In addition, changes in the expression of genes controlling the integrity of the intestinal barrier (*Duox, Upd3, PPO2*, and *Hml*) were noted after exposure to CuONP (Alaraby, Hernández, and Marcos 2015d), and for this reason, it was proposed as a reliable model to mimic what might occur in mammalian intestine after exposure to NM.

In the human intestine there are specific gut microbiota that are affected by different external agents including NM. Imbalances of microbiota have been associated with different human diseases. In addition to Crohn's disease and ulcerative colitis, other diseases such as diabetes, metabolic syndrome, nonalcoholic fatty liver disease or steatohepatitis, and even psychiatric disorders such as depression, and multiple sclerosis have been correlated to altered microbiota (Biedermann and Rogler 2015). *Drosophila* also possess specific microbiota with important roles in host physiology and pathology (Kuraishi, Hori, and Kurata 2013; Lee and Brey 2013), and changes in the gut microbiota influence the health status of *Drosophila* including development, growth and body size (Shin et al. 2011). These observations open a new field to study harmful effects of NM, as recent studies noted that AgNP exposure markedly reduced the diversity of the gut microbiota (Han et al. 2014), as occurs after exposure to CuNP (Alaraby, Hernández, and Marcos 2015d). These are only two examples of potential of *Drosophila* to assess adverse health effects associated with NM exposure.

According to the findings obtained in the available literature, one may conclude that *Drosophila melanogaster* offers a qualified in vivo model that succeeds in evaluating a wide set of different NM utilizing various approaches:

(1) Mimicking NP internalization routes (oral administration or inhalation).
(2) Evaluating stress and apoptosis responses after NP exposure via expression changes in *Hsps, p53, caspase 3, caspase 9*, and others.
(3) Assessing oxidative stress due to NP exposure via ROS production and antioxidant enzymes activity.
(4) Determining genotoxicity and level of DNA damage resulting from NP exposure, by comet and SMART assay (exclusive to *Drosophila*).
(5) Detecting NP toxicity (viability, life span, fertility, and fecundity).
(6) Assessing morphological abnormalities related to NP exposure as loss of pigmentation and altered structure and/or functions of different tissues.
(7) Detecting neural malfunction related to NP exposure.
(8) Detecting behavioral effect of NM exposure, such as grooming mechanisms, developmental fitness.
(9) Determining physiological and metabolic malfunction after NP exposure.
(10) Showing NM effects at different life stages, from embryo to adult stages, and over multiple generations.



Q15 **Figure 5.** Representative pictures following the oral administration pathway and nanoparticles internalization. *Drosophila melanogaster* larval stage (A) is usually exposed to nanomaterials, as is the case of Ce-NP (B). The nanomaterial (Ce-NP) is observed inside the midgut lumen, attached to microvilli (D), as well as internalized inside midgut cells of the *Drosophila* larva (E). Arrows indicate Ce-NP.

Funding

This investigation has been partially supported by the Generalitat de Catalunya (CIRIT, 2014SGR-205), Barcelona (Spain), and by the Ministry of Economy and Competition SAF2015-63519-R. M. Alaraby held a fellowship from the Cultural Affairs Sector and Missions (Ministry of Higher Education), Egypt. B. Annangi was supported by a postdoctoral fellowship from the Universitat Autònoma de Barcelona (UAB).

Conflict of interest

The authors declare that there is no conflict of interest.

References

Abdalaziz, M. A., B. Annangi, and R. Marcos. 2014. Testing the genotoxic potential of nanomaterials using *Drosophila*. In *Genotoxicity and DNA repair*, 297–304. New York, NY: Springer. Q5

Abolaji, A. O., J. P. Kamdem, E. O. Farombi, and J. B. T. Rocha. 2013. *Drosophila melanogaster* as a promising model organism in toxicological studies. *Archives of Basic and Applied Medicine* 1:33–38. Q4

Adam, N., A. Vakurov, D. Knapen, and R. Blust. 2015. The chronic toxicity of CuO nanoparticles and copper salt to *Daphnia magna*. *Journal of Hazardous Materials* 283:416–22. doi:10.1016/j.jhazmat.2014.09.037.

Adams, M. D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne, P. G. Amanatides, S. E. Scherer, P. W. Li, R. A. Hoskins, R. F. Galle, R. A. George, S. E. Lewis, S. Richards, M. Ashburner, S. N. Henderson, G. G. Sutton, J. R. Wortman, M. D. Yandell, Q. Zhang, L. X. Chen, R. C. Brandon, Y. H. Rogers, R. G. Blazej, M. Champe, B. D. Pfeiffer, K. H. Wan, C. Doyle, E. G. Baxter, G. Helt, C. R. Nelson, G. L. Gabor, J. F. Abril, A. Agbayani, H. J. An, C. Andrews-Pfannkoch, D. Baldwin, R. M. Ballew, A. Basu, J. Baxendale, L. Bayraktaroglu, E. M. Beasley, K. Y. Beeson, P. V. Benos, B. P. Berman, D. Bhandari, S. Bolshakov, D. Borkova, M. R. Botchan, J. Bouck, P. Brokstein, P. Brottier, K. C. Burtis, D. A. Busam, H. Butler, E. Cadieu, A. Center, I. Chandra, J. M. Cherry, S. Cawley, C. Dahlke, L. B. Davenport, P. Davies, B. De Pablos, A. Delcher, Z. Deng, A. D. Mays, I. Dew, S. M. Dietz, K. Dodson, L. E. Doup, M. Downes, S. Dugan-Rocha, B. C. Dunkov, P. Dunn, K. J. Durbin, C. C. Evangelista, C. Ferraz, S. Ferriera, W. Fleischmann, C. Fosler, A. E. Gabrielian, N. S. Garg, W. M. Gelbart, K. Glasser, A. Glodek, F. Gong, J. H. Gorrell, Z. Gu, P. Guan, M. Harris, N. L. Harris, D. Harvey, T. J. Heiman, J. R. Hernandez, J. Houck, D. Hostin, K. A. Houston, T. J. Howland, M. H. Wei, C. Ibegwam, M. Jalali, F. Kalush, G. H. Karpen, Z. Ke, J. A. Kennison, K. A. Ketchum, B. E. Kimmel, C. D. Kodira, C. Kraft, S. Kravitz, D. Kulp, Z. Lai, P. Lasko, Y. Lei, A. A. Levitsky, J. Li, Z. Li, Y. Liang, X. Lin, X. Liu, B. Mattei, T. C. McIntosh, M. P. McLeod, D. McPherson, G. Merkulov, N. V. Milshina, C. Mobarry, J. Morris, A. Moshrefi, S. M. Mount, M. Moy, B. Murphy, L. Murphy, D. M. Muzny, D. L. Nelson, D. R. Nelson, K. A. Nelson, K. Nixon, D. R. Nusskern, J. M. Pacleb, M. Palazzolo, G. S. Pittman, S. Pan, J. Pollard, V. Puri, M. G. Reese, K. Reinert, K. Remington, R. D. Saunders, F. Scheeler, H. Shen, B. C. Shue, I. Sidén-Kiamos, M. Simpson, M. P. Skupski, T. Smith, E. Spier, A. C. Spradling, M. Stapleton, R. Strong, E. Sun, R. Svirskas, C. Tector, R. Turner, E. Venter, A. H. Wang, X. Wang, Z. Y. Wang, D. A. Wassarman, G. M. Weinstock, J. Weissenbach, S. M. Williams, T. Woodage, K. C. Worley, D. Wu, S. Yang, Q. A. Yao, J. Ye, R. F. Yeh, J. S. Zaveri, M. Zhan, G. Zhang, Q. Zhao, L. Zheng, X. H. Zheng, F. N. Zhong, W. Zhong, X. Zhou, S. Zhu, X. Zhu, H. O. Smith, R. A. Gibbs, E. W. Myers, G. M. Rubin, and J. C. Venter. 2000. The genome sequence of *Drosophila melanogaster*. *Science* 287:2185–95. doi:10.1126/science.287.5461.2185.

Adolfsson, K., M. Schneider, G. Hammarin, U. Häcker, and C. N. Prinz. 2013. Ingestion of gallium phosphide nanowires has no adverse effect on *Drosophila* tissue function. *Nanotechnology* 24:285101. doi:10.1088/0957-4484/24/28/285101.

Ahamed, M., R. Posgai, T. J. Gorey, M. Nielsen, S. M. Hussain, and J. J. Rowe. 2010a. Silver nanoparticles induced heat shock protein 70, oxidative stress and apoptosis in *Drosophila melanogaster*. *Toxicology and Applied Pharmacology* 242:263–69. doi:10.1016/j.taap.2009.10.016.

Ahamed, M., M. A. Siddiqui, M. J. Akhtar, I. Ahmad, A. B. Pant, and H. A. Alhadlaq. 2010b. Genotoxic potential of copper oxide nanoparticles in human lung epithelial cells. *Biochemical and Biophysical Research Communications* 396:578–83. doi:10.1016/j.bbrc.2010.04.156.

Aillon, K. L., Y. Xie, N. El-Gendy, C. J. Berkland, and M. L. Forrest. 2009. Effects of nanomaterial physicochemical properties on *in vivo* toxicity. *Advanced Drug Delivery Reviews* 61:457–66. doi:10.1016/j.addr.2009.03.010.

Akhtar, M. J., S. Kumar, H. A. Alhadlaq, S. A. Alrokayan, K. M. Abu-Salah, and M. Ahamed. 2013. Dose-dependent genotoxicity of copper oxide nanoparticles stimulated by reactive oxygen species in human lung epithelial cells. *Toxicology and Industrial Health*. doi:10.1177/0748233713511512. Q14

Alaraby, M., B. Annangi, A. Hernández, A. Creus, and R. Marcos. 2015a. A comprehensive study of the harmful effects of ZnO nanoparticles using *Drosophila melanogaster* as an *in vivo* model. *Journal of Hazardous Materials* 296:166–74. doi:10.1016/j.jhazmat.2015.04.053.

Alaraby, M., E. Demir, A. Hernández, and R. Marcos. 2015b. Assessing potential harmful effects of CdSe quantum dots by using *Drosophila melanogaster* as *in vivo* model. *Science of The Total Environment* 530–31:66–75. doi:10.1016/j.scitotenv.2015.05.069.

Alaraby, M., A. Hernández, B. Annangi, E. Demir, J. Bach, L. Rubio, A. Creus, and R. Marcos. 2015c. Antioxidant and antigenotoxic properties of CeO_2 NPs and cerium sulphate: Studies with *Drosophila melanogaster* as a promising *in vivo* model. *Nanotoxicology* 9:749–59. doi:10.3109/17435390.2014.976284.

Alaraby, M., A. Hernández, and R. Marcos. 2015d. New insights in the toxic/genotoxic effects of CuO nanoparticles in the *in vivo Drosophila* model. *Nanotoxicology*. doi:10.3109/17435390.2015.1121413. Q15

Ali, D., S. Alarifi, S. Alkahtani, A. A. AlKahtane, and A. Almalik. 2015. Cerium oxide nanoparticles induce oxidative stress and genotoxicity in human skin melanoma cells. *Cell Biochemistry and Biophysics* 71:1643–51. doi:10.1007/s12013-014-0386-6.

Alkilany, A. M., and C. J. Murphy. 2010. Toxicity and cellular uptake of gold nanoparticles: What we have learned so far? *Journal of Nanoparticle Research* 12:2313–33. doi:10.1007/s11051-010-9911-8.

Ambegaokar, S. S., B. Roy, and G. R. Jackson. 2010. Neurodegenerative models in *Drosophila*: Polyglutamine disorders, Parkinson disease, and amyotrophic lateral sclerosis. *Neurobiology of Disease* 40:29–39. doi:10.1016/j.nbd.2010.05.026.

Annangi, B., J. Bach, G. Vales, L. Rubio, R. Marcos, and A. Hernández. 2015b. Long-term exposures to low doses of cobalt nanoparticles induce cell transformation enhanced by oxidative damage. *Nanotoxicology* 9:138–47. doi:10.3109/17435390.2014.900582.

Annangi, B., L. Rubio, M. Alaraby, J. Bach, R. Marcos, and A. Hernández. 2015a. Acute and long-term in vitro effects of zinc oxide nanoparticles. *Archives of Toxicology*. [Epub ahead of print].

Arami, H., A. Khandhar, D. Liggitt, and K. M. Krishnan. 2015. In vivo delivery, pharmacokinetics, biodistribution Q6

and toxicity of iron oxide nanoparticles. *Chemical Society Reviews* 44:8576–607. doi:10.1039/C5CS00541H.

Armstrong, N., M. Ramamoorthy, D. Lyon, K. Jones, and A. Duttaroy. 2013. Mechanism of silver nanoparticles action on insect pigmentation reveals intervention of copper homeostasis. *PloS ONE* 8:e53186. doi:10.1371/journal.pone.0053186.

Asakura, M., T. Sasaki, T. Sugiyama, M. Takaya, S. Koda, K. Nagano, H. Arito, and S. Fukushima. 2010. Genotoxicity and cytotoxicity of multi-wall carbon nanotubes in cultured Chinese hamster lung cells in comparison with chrysotile A fibers. *Journal of Occupational Health* 52:155–66. doi:10.1539/joh.L9150.

AshaRani, P. V., G. Low Kah Mun, M. P. Hande, and S. Valiyaveettil. 2008. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano* 3:279–90. doi:10.1021/nn800596w.

Austin, L. A., M. A. Mackey, E. C. Dreaden, and M. A. El-Sayed. 2014. The optical, photothermal, and facile surface chemical properties of gold and silver nanoparticles in biodiagnostics, therapy, and drug delivery. *Archives of Toxicology* 88:1391–417. doi:10.1007/s00204-014-1245-3.

Avalos, A., A. I. Haza, E. Drosopoulou, P. Mavragani-Tsipidou, and P. Morales. 2015. In vivo genotoxicity assesment of silver nanoparticles of different sizes by the Somatic Mutation and Recombination Test (SMART) on *Drosophila*. *Food and Chemical Toxicology* 85:114–19. doi:10.1016/j.fct.2015.06.024.

Baeza, A., and M. Vallet-Regí. 2015. Smart mesoporous silica nanocarriers for antitumoral therapy. *Current Topics Medica Chemical* 15:2306–15. doi:10.2174/1568026615666150605114826.

Balakrishnan, V., H. A. Ab Wab, K. A. Razak, and S. Shamsuddin. 2013. In vitro evaluation of cytotoxicity of colloidal amorphous silica nanoparticles designed for drug delivery on human cell lines. *Journal of Nanomaterials* 2013:4.

Barandeh, F., P. L. Nguyen, R. Kumar, G. J. Iacobucci, M. L. Kuznicki, A. Kosterman, E. J. Bergeyn, P. N. Prasad, and S. Gunawardena. 2012. Organically modified silica nanoparticles are biocompatible and can be targeted to neurons in vivo. *PLoS ONE* 7:e29424. doi:10.1371/journal.pone.0029424.

Barnes, C. A., A. Elsaesser, J. Arkusz, A. Smok, J. Palus, A. Lesniak, A. Salvati, J. P. Hanrahan, W. H. Jong, E. Dziubałtowska, M. Stepnik, K. Rydzyński, G. McKerr, I. Lynch, K. A. Dawson, and C. V. Howard. 2008. Reproducible comet assay of amorphous silica nanoparticles detects no genotoxicity. *Nano Letters* 8:3069–74. doi:10.1021/nl801661w.

Bharali, D. J., I. Klejbor, E. K. Stachowiak, P. Dutta, I. Roy, N. Kaur, E. J. Bergey, P. N. Prasad, and M. K. Stachowiak. 2005. Organically modified silica nanoparticles: A nonviral vector for *in vivo* gene delivery and expression in the brain. *Proceedings of the National Academy of Sciences* 102:11539–44. doi:10.1073/pnas.0504926102.

Bianco, A., K. Kostarelos, and M. Prato. 2005. Applications of carbon nanotubes in drug delivery. *Current Opinion in Chemical Biology* 9:674–79. doi:10.1016/j.cbpa.2005.10.005.

Biedermann, L., and G. Rogler. 2015. The intestinal microbiota: Its role in health and disease. *European Journal of Pediatrics* 174:151–67. doi:10.1007/s00431-014-2476-2.

Bilen, J., and N. M. Bonini. 2005. *Drosophila* as a model for human neurodegenerative disease. *Annual Review of Genetics* 39:153–71. doi:10.1146/annurev.genet.39.110304.095804.

Bishop, A. J., and R. H. Schiestl. 2003. Role of homologous recombination in carcinogenesis. *Experimental and Molecular Pathology* 74:94–105. doi:10.1016/S0014-4800(03)00010-8.

Braz, M. G., J. P. De Castro Marcondes, M. A. Matsumoto, M. H. Duarte, D. M. F. Salvadori, and D. A. Ribeiro. 2008. Genotoxicity in primary human peripheral lymphocytes after exposure to radiopacifiers *in vitro*. *Journal Materials Science Materials in Medicine* 19:601–05.

Bruchez, M., M. Moronne, P. Gin, S. Weiss, and A. P. Alivisatos. 1998. Semiconductor nanocrystals as fluorescent biological labels. *Science* 281:2013–16. doi:10.1126/science.281.5385.2013.

Brunetti, V., H. Chibli, R. Fiammengo, A. Galeone, M. A. Malvindi, G. Vecchio, R. Cingolani, J. L. Nadeau, and P. P. Pompa. 2013. InP/ZnS as a safer alternative to CdSe/ZnS core/shell quantum dots: *In vitro* and *in vivo* toxicity assessment. *Nanoscale* 5:307–17.

Buerki-Thurnherr, T., L. Xiao, L. Diener, O. Arslan, C. Hirsch, X. Maeder-Althaus, K. Grieder, B. Wampfler, S. Mathur, P. Wick, and H. F. Krug. 2013. *In vitro* mechanistic study towards a better understanding of ZnO nanoparticle toxicity. *Nanotoxicology* 7:402–16. doi:10.3109/17435390.2012.666575.

Carmona, E. R., B. Escobar, G. Vales, and R. Marcos. 2015a. Genotoxic testing of titanium dioxide anatase nanoparticles using the wing-spot test and the comet assay in *Drosophila*. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 778:12–21. doi:10.1016/j.mrgentox.2014.12.004.

Carmona, E. R., T. N. Guecheva, A. Creus, and R. Marcos. 2011. Proposal of an *in vivo* comet assay using haemocytes of *Drosophila melanogaster*. *Environmental and Molecular Mutagenesis* 52:165–69. doi:10.1002/em.v52.2.

Carmona, E. R., C. Inostroza-Blancheteau, L. Rubio, and R. Marcos. 2015b. Genotoxic and oxidative stress potential of nanosized and bulk zinc oxide particles in *Drosophila melanogaster*. *Toxicology and Industrial Health*. Pii:0748233715599472. Q7

Carmona, E. R., C. Inostroza-Blancheteau, L. Rubio, and R. Marcos. 2015c. Genotoxic effects of copper oxide nanoparticles in *Drosophila melanogaster*. *Mutation Research* 791:1–11. doi:10.1016/j.mrgentox.2015.07.006.

Celardo, I., J. Z. Pedersen, E. Traversa, and L. Ghibelli. 2011. Pharmacological potential of cerium oxide nanoparticles. *Nanoscale* 3:1411–20. doi:10.1039/c0nr00875c.

Charroux, B., and J. Royet. 2012. Gut-microbiota interactions in non-mammals: What can we learn from *Drosophila*? *Seminars in Immunology* 24:17–24. doi:10.1016/j.smim.2011.11.003.

Chatterjee, N., H. J. Eom, and J. Choi. 2014b. Effects of silver nanoparticles on oxidative DNA damage-repair as a

function of p38 MAPK status: A comparative approach using human Jurkat T cells and the nematode *Caenorhabditis elegans*. *Environmental and Molecular Mutagenesis* 55:122–33. doi:10.1002/em.v55.2.

Chatterjee, N., J. Yang, H.-M. Kim, E. Jo, P.-J. Kim, K. Choi, and J. Choi. 2014a. Potential Toxicity of differential functionalized multiwalled carbon nanotubes (MWCNT) in human cell line (BEAS2B) and *Caenorhabditis elegans*. *Journal of Toxicology and Environmental Health, Part A* 77:1399–408. doi:10.1080/15287394.2014.951756.

Chen, H., B. Wang, W. Feng, W. Du, H. Ouyang, Z. Chai, and X. Bi. 2015. Oral magnetite nanoparticles disturb the development of *Drosophila melanogaster* from oogenesis to adult emergence. *Nanotoxicology* 9:302–12. doi:10.3109/17435390.2014.929189.

Chen, J., X. Dong, J. Zhao, and G. Tang. 2009. *In vivo* acute toxicity of titanium dioxide nanoparticles to mice after intraperitioneal injection. *Journal of Applied Toxicology* 29:330–37. doi:10.1002/jat.v29:4.

Chen, T., J. Yan, and Y. Li. 2014. Genotoxicity of titanium dioxide nanoparticles. *Journal of Food and Drug Analysis* 22:95–104. doi:10.1016/j.jfda.2014.01.008.

Chin, C. D., T. Laksanasopin, Y. K. Cheung, D. Steinmiller, V. Linder, H. Parsa, J. Wang, H. Moore, R. Rouse, G. Umviligihozo, E. Karita, L. Mwambarangwe, S. L. Braunstein, J. Van De Wijgert, R. Sahabo, J. E. Justman, W. El-Sadr, and S. K. Sia. 2011. Microfluidics-based diagnostics of infectious diseases in the developing world. *Nature Medicine* 17:1015–19. doi:10.1038/nm.2408.

Choi, H.-S., Y.-J. Kim, M. Song, M.-K. Song, and J.-C. Ryu. 2011. Genotoxicity of nano-silica in mammalian cell lines. *Toxicogical Environment Health Sciences* 3:7–13. doi:10.1007/s13530-011-0072-7.

Clift, M. J. D., M. S. P. Boyles, D. M. Brown, and V. Stone. 2010. An investigation into the potential for different surface-coated quantum dots to cause oxidative stress and affect macrophage cell signaling *in vitro*. *Nanotoxicology* 4:139–49. doi:10.3109/17435390903276925.

Cohen, C. A., J. A. Karfakis, M. D. Kurnick, and B. Rzigalinski. 2008. Cerium oxide nanoparticles reduce free radical-mediated toxicity in *Drosophila melanogaster*. *FASEB Journal* 22: 624–1. (abstract).

Contado, C. 2015. Nanomaterials in consumer products: A challenging analytical problem. *Frontiers Chemical* 3:48. doi:10.3389/fchem.2015.00048.

Contreras, E. Q., M. Cho, H. Zhu, H. L. Puppala, G. Escalera, W. Zhong, and V. L. Colvin. 2012. Toxicity of quantum dots and cadmium salt to *Caenorhabditis elegans* after multigenerational exposure. *Environmental Science & Technology* 47:1148–54. doi:10.1021/es3036785.

Corma, A., P. Atienzar, H. Garcia, and J.-Y. Chane-Ching. 2004. Hierarchically meso structured doped CeO_2 with potential for solar-cell use. *Nature Materials* 3:394–97. doi:10.1038/nmat1129.

Couvreur, P., G. Barratt, E. Fattal, and C. Vauthier. 2002. Nanocapsule technology: A review. *Critical Reviews in Therapeutic Drug Carrier Systems* 19:99–134. doi:10.1615/CritRevTherDrugCarrierSyst.v19.i2.

Dabbousi, B. O., J. Rodriguez-Viejo, F. V. Mikulec, J. R. Heine, H. Mattoussi, R. Ober, K. F. Jensen, and M. G. Bawendi. 1997. (CdSe)ZnS Core–Shell Quantum Dots: Synthesis and Characterization of a Size Series of Highly Luminescent Nanocrystallites. *Journal of Physical Chemistry. B* 101:9463–75. doi:10.1021/jp971091y.

Das, S., J. M. Dowding, K. E. Klump, J. F. McGinnis, W. Self, and S. Seal. 2013. Cerium oxide nanoparticles: Applications and prospects in nanomedicine. *Nanomedicine (London)* 8:1483–508. doi:10.2217/nnm.13.133.

de Andrade, L. R., A. S. Brito, A. M. G. de Souza Melero, H. Zanin, H. J. Ceragioli, V. Baranauskas, K. S. Cunha, and S. P. Irazusta. 2014. Absence of mutagenic and recombinagenic activity of multi-walled carbon nanotubes in the *Drosophila* wing-spot test and *Allium cepa* test. *Ecotoxicology and Environmental Safety* 99:92–97. doi:10.1016/j.ecoenv.2013.10.013.

Dedeh, A., A. Ciutat, M. Treguer-Delapierre, and J.-P. Bourdineaud. 2015. impact of gold nanoparticles on zebrafish exposed to a spiked sediment. *Nanotoxicology* 9:71–80. doi:10.3109/17435390.2014.889238.

Demir, E., S. Aksakal, F. Turna, B. Kaya, and R. Marcos. 2015. *In vivo* genotoxic effects of four different nano-sizes forms of silica nanoparticles in *Drosophila melanogaster*. *Journal of Hazardous Materials* 283:260–66. doi:10.1016/j.jhazmat.2014.09.029.

Demir, E., D. Burgucu, F. Turna, S. Aksakal, and B. Kaya. 2013b. Determination of TiO_2, ZrO_2, and Al_2O_3 nanoparticles on genotoxic responses in human peripheral blood lymphocytes and cultured embryonic kidney cells. *Journal of Toxicology and Environmental Health, Part A* 76:990–100. doi:10.1080/15287394.2013.830584.

Demir, E., A. Creus, and R. Marcos. 2014. Genotoxicity and DNA repair processes of zinc oxide nanoparticles. *Journal of Toxicology and Environmental Health, Part A* 77:1292–303. doi:10.1080/15287394.2014.935540.

Demir, E., F. Turna, G. Vales, B. Kaya, A. Creus, and R. Marcos. 2013a. *In vivo* genotoxicity assessment of titanium, zirconium and aluminium nanoparticles, and their microparticulated forms, in *Drosophila*. *Chemosphere* 93:2304–10. doi:10.1016/j.chemosphere.2013.08.022.

Demir, E., G. Vales, B. Kaya, A. Creus, and R. Marcos. 2011. Genotoxic analysis of silver nanoparticles in *Drosophila*. *Nanotoxicology* 5:417–24. doi:10.3109/17435390.2010.529176.

Dobrzyńska, M. M., A. Gajowik, J. Radzikowska, A. Lankoff, M. Dušinská, and M. Kruszewski. 2014. Genotoxicity of silver and titanium dioxide nanoparticles in bone marrow cells of rats *in vivo*. *Toxicology* 315:86–91. doi:10.1016/j.tox.2013.11.012.

Dong, J., and Q. Ma. 2015. Suppression of basal and carbon nanotube-induced oxidative stress, inflammation and fibrosis in mouse lungs by Nrf2. *Nanotoxicology* 1–11. [Epub ahead of print]. doi:10.3109/17435390.2015.1110758. Q16

Dreher, K. L. 2004. Health and environmental impact of nanotechnology: Toxicological assessment of manufactured nanoparticles. *Toxicological Sciences* 77:3–5. doi:10.1093/toxsci/kfh041.

Dresselhaus, M. S., G. Dresselhaus, and A. Jorio. 2004. Unusual properties and structure of carbon nanotubes. *Annual Review of Materials Research* 34:247–78. doi:10.1146/annurev.matsci.34.040203.114607.

Eom, H.-J., and J. Choi. 2009. Oxidative stress of CeO_2 nanoparticles via p38-Nrf-2 signaling pathway in human bronchial epithelial cell, Beas-2B. *Toxicology Letters* 187:77–83. doi:10.1016/j.toxlet.2009.01.028.

Foriel, S., P. Willems, J. Smeitink, A. Schenck, and J. Beyrath. 2015. Mitochondrial diseases: *Drosophila melanogaster* as a model to evaluate potential therapeutics. *International Journal of Biochemistry & Cell Biology* 63:60–65. doi:10.1016/j.biocel.2015.01.024.

Fruijtier-Pölloth, C. 2012. The toxicological mode of action and the safety of synthetic amorphous silica-a nanostructured material. *Toxicology* 294:61–79. doi:10.1016/j.tox.2012.02.001.

Gagné, F., J. Auclair, P. Turcotte, M. Fournier, C. Gagnon, S. Sauvé, and C. Blaise. 2008. Ecotoxicity of CdTe quantum dots to freshwater mussels: Impacts on immune system, oxidative stress and genotoxicity. *Aquatic Toxicology* 86:333–40. doi:10.1016/j.aquatox.2007.11.013.

Gagne, F., J. Auclair, P. Turcotte, and C. Gagnon. 2013. Sublethal effects of silver nanoparticles and dissolved silver in freshwater mussels. *Journal of Toxicology and Environmental Health, Part A* 76:479–90. doi:10.1080/15287394.2013.779561.

Galeone, A., G. Vecchio, M. A. Malvindi, V. Brunetti, R. Cingolani, and P. P. Pompa. 2012. In vivo assessment of CdSe–ZnS quantum dots: Coating dependent bioaccumulation and genotoxicity. *Nanoscale* 4:6401–07. doi:10.1039/c2nr31826a.

Gebel, T., H. Foth, G. Damm, A. Freyberger, P.-J. Kramer, W. Lilienblum, C. Röhl, T. Schupp, C. Weiss, K.-M. Wollin, and J. G. Hengstler. 2014. Manufactured nanomaterials: Categorization and approaches to hazard assessment. *Archives of Toxicology* 88:2191–211. doi:10.1007/s00204-014-1383-7.

Giljohann, D. A., D. S. Seferos, W. L. Daniel, M. D. Massich, P. C. Patel, and C. A. Mirkin. 2010. Gold nanoparticles for biology and medicine. *Angewandte Chemie International Edition* 49:3280–94. doi:10.1002/anie.200904359.

Godwin, H., C. Nameth, D. Avery, L. L. Bergeson, D. Bernard, E. Beryt, W. Boyes, S. Brown, A. J. Clippinger, Y. Cohen, M. Doa, C. O. Hendren, P. Holden, K. Houck, A. B. Kane, F. Klaessig, T. Kodas, R. Landsiedel, I. Lynch, T. Malloy, M. B. Miller, J. Muller, G. Oberdorster, E. J. Petersen, R. C. Pleus, P. Sayre, V. Stone, K. M. Sullivan, J. Tentschert, P. Wallis, and E. Nel. 2015. Nanomaterial categorization for assessing risk potential to facilitate regulatory decision-making. *ACS Nano* 9:3409–17. doi:10.1021/acsnano.5b00941.

Golbamaki, N., B. Rasulev, A. Cassano, R. L. Marchese Robinson, E. Benfenati, J. Leszczynski, and M. T. D. Cronin. 2015. Genotoxicity of metal oxide nanomaterials: Review of recent data and discussion of possible mechanisms. *Nanoscale* 7:2154–98. doi:10.1039/C4NR06670G.

Gomes, T., O. Araujo, R. Pereira, A. C. Almeida, A. Cravo, and M. J. Bebianno. 2013. Genotoxicity of copper oxide and silver nanoparticles in the mussel *Mytilus galloprovincialis*. *Marine Environmental Research* 84:51–59. doi:10.1016/j.marenvres.2012.11.009.

Gonzalez, C. 2013. *Drosophila melanogaster*: A model and a tool to investigate malignancy and identify new therapeutics. *Nature Reviews Cancer* 13:172–83. doi:10.1038/nrc3461.

Gorth, D. J., D. M. Rand, and T. J. Webster. 2011. Silver nanoparticle toxicity in *Drosophila*: Size does matter. *International Journal Nanomed* 6:343–50.

Haberl, N., S. Hirn, A. Wenk, J. Diendorf, M. Epple, B. D. Johnston, F. Krombach, W. G. Kreyling, and C. Schleh. 2013. Cytotoxic and proinflammatory effects of PVP-coated silver nanoparticles after intratracheal instillation in rats. *Beilstein Journal of Nanotechnology* 4:933–40. doi:10.3762/bjnano.4.105.

Hadrup, N., A. K. Sharma, M. Poulsen, and E. Nielsen. 2015. Toxicological risk assessment of elemental gold following oral exposure to sheets and nanoparticles—A review. *Regulatory Toxicology and Pharmacology* 72:216–21. doi:10.1016/j.yrtph.2015.04.017.

Han, X., B. Geller, K. Moniz, P. Das, A. K. Chippindale, and V. K. Walker. 2014. Monitoring the developmental impact of copper and silver nanoparticle exposure in *Drosophila* and their microbiomes. *Science of the Total Environment* 487:822–29. doi:10.1016/j.scitotenv.2013.12.129.

Handy, R. D., N. Van Den Brink, M. Chappell, M. Mühling, R. Behra, M. Dušinská, P. Simpson, J. Ahtiainen, A. N. Jha, J. Seiter, A. Bednar, A. Kennedy, T. F. Fernandes, and M. Riediker. 2012. Practical considerations for conducting ecotoxicity test methods with manufactured nanomaterials: What have we learnt so far? *Ecotoxicology* 21:933–72. doi:10.1007/s10646-012-0862-y.

Hartmann, N. B., K. A. Jensen, A. Baun, K. Rasmussen, H. Rauscher, R. Tantra, D. Cupi, D. Gilliland, F. Pianella, and J. M. Riego Sintes. 2015. Techniques and protocols for dispersing nanoparticle powders in aqueous media—Is there a rationale for harmonization? *Journal of Toxicology and Environmental Health, Part B* 18:299–326. doi:10.1080/10937404.2015.1074969.

Hawkins, A. D., A. D. Hawkins, C. Thornton, A. J. Kennedy, K. Bu, J. Cizdziel, B. W. Jones, J. A. Steevens, and K. L. Willett. 2015. Gill histopathologies following exposure to nanosilver or silver nitrate. *Journal of Toxicology and Environmental Health, Part A* 78:301–15. doi:10.1080/15287394.2014.971386.

Hayakawa, T., Y. Shitomi, K. Miyamoto, and H. Hori. 2004. GalNAc pretreatment inhibits trapping of *Bacillus thuringiensis* Cry1Ac on the peritrophic membrane of *Bombyx mori*. *FEBS Letters* 576:331–35. doi:10.1016/j.febslet.2004.09.029.

Hayashi, Y., P. Engelmann, R. Foldbjerg, M. Szabó, I. Somogyi, E. Pollák, L. Molnár, H. Autrup, D. S. Sutherland, J. Scott-Fordsmand, and L.-H. Heckmann. 2012. Earthworms and humans *in vitro*: Characterizing evolutionarily conserved stress and immune responses to silver nanoparticles. *Environmental Science & Technology* 46:4166–73. doi:10.1021/es3000905.

He, X., W. G. Aker, and H.-M. Hwang. 2014. An *in vivo* study on the photo-enhanced toxicities of S-doped TiO_2 nanoparticles to zebrafish embryos (*Danio rerio*) in terms of malformation, mortality, rheotaxis dysfunction, and DNA damage. *Nanotoxicology* 8:185–95. doi:10.3109/17435390.2013.874050.

Hegedus, D., M. Erlandson, C. Gillott, and U. Toprak. 2009. New insights into peritrophic matrix synthesis, architecture, and function. *Annual Review of Entomology* 54:285–302. doi:10.1146/annurev.ento.54.110807.090559.

Hirsch, A. 2002. Functionalization of single-walled carbon nanotubes. *Angewandte Chemie International Edition* 41:1853–59. doi:10.1002/1521-3773(20020603)41:11<1853::AID-ANIE1853>3.0.CO;2-N.

Huang, N., Y. Yan, Y. Xu, Y. Jin, J. Lei, X. Zou, D. Ran, H. Zhang, S. Luan, and H. Gu. 2013. Alumina nanoparticles alter rhythmic activities of local interneurons in the antennal lobe of *Drosophila*. *Nanotoxicology* 7:212–20. doi:10.3109/17435390.2011.648668.

Hunt, P. R., B. J. Marquis, K. M. Tyner, S. Conklin, N. Olejnik, B. C. Nelson, and R. L. Sprando. 2013. Nanosilver suppresses growth and induces oxidative damage to DNA in *Caenorhabditis elegans*. *Journal of Applied Toxicology : JAT* 33:1131–42. doi:10.1002/jat.v33.10.

Hussain, S. M., K. L. Hess, J. M. Gearhart, K. T. Geiss, and J. J. Schlager. 2005. *In vitro* toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicology in Vitro* 19:975–83. doi:10.1016/j.tiv.2005.06.034.

Iijima, S. 1991. Helical microtubules of graphitic carbon. *Nature* 354:56–58. doi:10.1038/354056a0.

Isani, G., M. L. Falcioni, G. Barucca, D. Sekar, G. Andreani, E. Carpenè, and G. Falcioni. 2013. Comparative toxicity of CuO nanoparticles and $CuSO_4$ in rainbow trout. *Ecotoxicology and Environmental Safety* 97:40–46. doi:10.1016/j.ecoenv.2013.07.001.

Izu, N., W. Shin, I. Matsubara, and N. Murayama. 2004. Development of resistive oxygen sensors based on cerium oxide thick film. *Journal of Electroceramics* 13:703–06. doi:10.1007/s10832-004-5179-7.

Ji, X., F. Peng, Y. Zhong, Y. Su, and Y. He. 2014. Fluorescent quantum dots: Synthesis, biomedical optical imaging, and biosafety assessment. *Colloids and Surfaces B: Biointerfaces* 124:132–39. doi:10.1016/j.colsurfb.2014.08.036.

Jia, G., H. Wang, L. Yan, X. Wang, R. Pei, T. Yan, Y. Zhao, and X. Guo. 2005. Cytotoxicity of carbon nanomaterials: Single-wall nanotube, multi-wall nanotube, and fullerene. *Environmental Science & Technology* 39:1378–83. doi:10.1021/es048729l.

Jing, X., J. H. Park, T. M. Peters, and P. S. Thorne. 2015. Toxicity of copper oxide nanoparticles in lung epithelial cells exposed at the air-liquid interface compared with in vivo assessment. *Toxicology in Vitro* 29:502–11. doi:10.1016/j.tiv.2014.12.023.

Jovanović, B., V. J. Cvetković, and T. L. Mitrović. 2015. Effects of human food grade titanium dioxide nanoparticle dietary exposure on *Drosophila melanogaster* survival, fecundity, pupation and expression of antioxidant genes. *Chemosphere* 144:43–49. doi:10.1016/j.chemosphere.2015.08.054.

Kannan, S. D., and G. M. Vijayaraghavan. 2014. Size-dependent effect of zinc oxide on toxicity and inflammatory potential of human monocytes. *Journal of Toxicology and Environmental Health, Part A* 77:177–91. doi:10.1080/15287394.2013.853224.

Karlsson, H. L., P. Cronholm, J. Gustafsson, and L. Moller. 2008. Copper oxide nanoparticles are highly toxic: A comparison between metal oxide nanoparticles and carbon nanotubes. *Chemical Research in Toxicology* 21:1726–32. doi:10.1021/tx800064j.

Karunakaran, G., R. Suriyaprabha, P. Manivasakan, R. Yuvakkumar, V. Rajendran, and N. Kannan. 2013. Screening of *in vitro* cytotoxicity, antioxidant potential and bioactivity of nano- and micro-ZrO_2 and -TiO_2 particles. *Ecotoxicology and Environmental Safety* 93:191–97. doi:10.1016/j.ecoenv.2013.04.004.

Kermanizadeh, A., I. Gosens, L. MacCalman, H. Johnston, P. H. Danielsen, N. R. Jacobsen, A.-G. Lenz, T. Fernandes, R. P. F. Schins, F. R. Cassee, H. Wallin, W. Kreyling, T. Stoeger, S. Loft, P. Moller, L. Tran, and V. Stone. 2016. A multi-laboratory toxicological assessment of a panel of 10 engineered nanomaterials to human health—ENPRA project—The highlights, limitations and current and future challenges. *Journal Toxicogical Environment Health B* 19: in press. Q8

Key, S. C. S., D. Reaves, F. Turner, and J. J. Bang. 2011. Impacts of silver nanoparticle ingestion on pigmentation and developmental progression in *Drosophila*. *Atlas Journal of Biology* 1:52–61. doi:10.5147/ajb.2011.0048.

Kim, J. S., E. Kuk, K. N. Yu, J. H. Kim, S. J. Park, H. J. Lee, S. H. Kim, Y. K. Park, Y. H. Park, C. Y. Hwang, Y. K. Kim, Y. S. Lee, D. H. Jeong, and M. H. Cho. 2007. Antimicrobial effects of silver nanoparticles. *Nanomedicine* 3:95–101.

Kim, J. S., and I. J. Yu. 2014. Single-wall carbon nanotubes (SWCNT) induce cytotoxicity and genotoxicity produced by reactive oxygen species (ROS) generation in phytohemagglutinin (PHA)-stimulated male human peripheral blood lymphocytes. *Journal of Toxicology and Environmental Health, Part A* 77:1141–53. doi:10.1080/15287394.2014.917062.

Kim, S.-H., and W.-J. Lee. 2014. Role of DUOX in gut inflammation: Lessons from *Drosophila* model of gut-microbiota interactions. *Front Cell Infect Microbiol* 3:116. doi:10.3389/fcimb.2013.00116.

Kuraishi, T., A. Hori, and S. Kurata. 2013. Host-microbe interactions in the gut of *Drosophila melanogaster*. *Frontiers in Physiology* 4:375. doi:10.3389/fphys.2013.00375.

Kwon, J. Y., P. Koedrith, and Y. R. Seo. 2014. Current investigations into the genotoxicity of zinc oxide and silica nanoparticles in mammalian models *in vitro* and *in vivo*: Carcinogenic/genotoxic potential, relevant mechanisms and biomarkers, artifacts, and limitations. *International Journal Nanomed* 9 (Suppl. 2):271–86.

Lee, W. J., and P. T. Brey. 2013. How microbiomes influence metazoan development: Insights from history and *Drosophila* modeling of gut-microbe interactions. *Annual Review of Cell and Developmental Biology* 29:571–92. doi:10.1146/annurev-cellbio-101512-122333.

Lee, Y.-G., J. Jeong, J. Raftis, and W.-S. Cho. 2015. Determination of adsorption affinity of nanoparticles for interleukin-8 secreted from A549 cells by *in vitro* cell-free and cell-based assays. *Journal of Toxicology and Environmental Health, Part A* 78:185–95. doi:10.1080/15287394.2014.955158.

Leeuw, T. K., R. M. Reith, R. A. Simonette, M. E. Harden, P. Cherukuri, D. Tsyboulski, K. M. Beckingham, and R. B. Weisman. 2007. Single-walled carbon nanotubes in the intact organism: near-IR imaging and biocompatibility studies in *Drosophila*. *Nano Letters* 7:2650–54. doi:10.1021/nl0710452.

Lei, R., C. Wu, B. Yang, H. Ma, C. Shi, Q. Wang, Q. Wang, Y. Yuan, and M. Liao. 2008. Integrated metabolomic analysis of the nano-sized copper particle-induced hepatotoxicity and nephrotoxicity in rats: A rapid *in vivo* screening method for nanotoxicity. *Toxicology and Applied Pharmacology* 232:292–301. doi:10.1016/j.taap.2008.06.026.

Lewinski, N., V. Colvin, and R. Drezek. 2008. Cytotoxicity of nanoparticles. *Small* 4:26–49. doi:10.1002/(ISSN)1613-6829.

Li, W.-R., X.-B. Xie, Q.-S. Shi, H.-Y. Zeng, Y.-S. Ou-Yang, and Y.-B. Chen. 2010. Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*. *Applied Microbiology and Biotechnology* 85:1115–22. doi:10.1007/s00253-009-2159-5.

Liao, M., and H. Liu. 2012. Gene expression profiling of nephrotoxicity from copper nanoparticles in rats after repeated oral administration. *Environmental Toxicology and Pharmacology* 34:67–80. doi:10.1016/j.etap.2011.05.014.

Lin, W., Y.-W. Huang, X.-D. Zhou, and Y. Ma. 2006. *In vitro* toxicity of silica nanoparticles in human lung cancer cells. *Toxicology and Applied Pharmacology* 217:252–59. doi:10.1016/j.taap.2006.10.004.

Liu, B., E. M. Campo, and T. Bossing. 2014b. *Drosophila* embryos as model to assess cellular and developmental toxicity of multi-walled carbon nanotubes (MWCNT) in living organisms. *PloS One* 9:e88681. doi:10.1371/journal.pone.0088681.

Liu, S., L. Xu, T. Zhang, G. Ren, and Z. Yang. 2010. Oxidative stress and apoptosis induced by nanosized titanium dioxide in PC12 cells. *Toxicology* 267:172–77. doi:10.1016/j.tox.2009.11.012.

Liu, X., C. Bu, Z. Nan, L. Zheng, Y. Qiu, and X. Lu. 2013a. Enzymes immobilized on amine-terminated ionic liquid-functionalized carbon nanotube for hydrogen peroxide determination. *Talanta* 105:63–68. doi:10.1016/j.talanta.2012.11.059.

Liu, X., H. Li, Q. Jin, and J. Ji. 2014a. Surface tailoring of nanoparticles via mixed-charge monolayers and their biomedical applications. *Small* 10:4230–42.

Liu, X., D. Vinson, D. Abt, R. H. Hurt, and D. M. Rand. 2009a. Differential toxicity of carbon nanomaterials in *Drosophila*: Larval dietary uptake is benign, but adult exposure causes locomotor impairment and mortality. *Environmental Science & Technology* 43:6357–63. doi:10.1021/es901079z.

Liu, Y., L. He, A. Mustapha, H. Li, Z. Q. Hu, and M. Lin. 2009b. Antibacterial activities of zinc oxide nanoparticles against *Escherichia coli* O157: H7. *Journal of Applied Microbiology* 107:1193–201. doi:10.1111/j.1365-2672.2009.04303.x.

Liu, Y., Y. Zhao, B. Sun, and C. Chen. 2013b. Understanding the toxicity of carbon nanotubes. *Accounts of Chemical Research* 46:702–13. doi:10.1021/ar300028m.

Lloyd, T. E., and J. P. Taylor. 2010. Flightless flies: *Drosophila* models of neuromuscular disease. *Annals of the New York Academy of Sciences* 1184:E1–20. doi:10.1111/j.1749-6632.2010.05432.x.

Lux Research. 2014. Nanotechnology update: Corporations up their spending as revenues for nano-enabled products increase. https://portal.luxresearchinc.com/research/report_excerpt/16215

Ma, N., C. Ma, C. Li, T. Wang, Y. Tang, H. Wang, X. Moul, Z. Chen, and N. Hel. 2013. Influence of nanoparticle shape, size, and surface functionalization on cellular uptake. *Journal of Nanoscience and Nanotechnology* 13:6485–98. doi:10.1166/jnn.2013.7525.

Machado, N. M., J. C. Lopes, R. S. Saturnino, E. B. Fagan, and J. C. Nepomuceno. 2013. Lack of mutagenic effect by multi-walled functionalized carbon nanotubes in the somatic cells of *Drosophila melanogaster*. *Food and Chemical Toxicology* 62:355–60. doi:10.1016/j.fct.2013.08.051.

Magaye, R., J. Zhao, L. Bowman, and M. Ding. 2012. Genotoxicity and carcinogenicity of cobalt-, nickel- and copper-based nanoparticles. *Experiments Theoretical Medica* 4:551–661.

Magdolenova, Z., A. Collins, A. Kumar, A. Dhawan, V. Stone, and M. Dusinska. 2014. Mechanisms of genotoxicity. A review of in vitro and in vivo studies with engineered nanoparticles. *Nanotoxicology* 8:233–78. doi:10.3109/17435390.2013.773464.

Manna, S. K., S. Sarkar, J. Barr, K. Wise, E. V. Barrera, O. Jejelowo, A. C. Rice-Ficht, and G. T. Ramesh. 2005. Single-walled carbon nanotube induces oxidative stress and activates nuclear transcription factor-κB in human keratinocytes. *Nano Letters* 5:1676–84. doi:10.1021/nl0507966.

Manshian, B. B., S. J. Soenen, A. Brown, N. Hondow, J. Wills, G. J. Jenkins, and S. H. Doak. 2016. Genotoxic capacity of Cd/Se semiconductor quantum dots with differing surface chemistries. *Mutagenesis* 31:97–106.

Marcos, R., and E. R. Carmona. 2013. The wing-spot and the comet tests as useful assays detecting genotoxicity in *Drosophila*. *Meth Molecular Biologic* 1044:417–27.

Massarsky, A., V. L. Trudeau, and T. W. Moon. 2014. Predicting the environmental impact of nanosilver. *Environmental Toxicology and Pharmacology* 38:861–73. doi:10.1016/j.etap.2014.10.006.

Maximino, C., R. X. Silva, S. N. Da Silva, L. S. Rodrigues, H. Barbosa, T. S. De Carvalho, L. K. Leão, M. G. Lima, K. R. Oliveira, and A. M. Herculano. 2015. Non-mammalian models in behavioral neuroscience: Consequences for biological psychiatry. *Frontiers in Behavioral Neuroscience* 9:233.

Maynard, A. D. 2006. *Nanotechnology: A research strategy for addressing risk. PEN 3*. Washington, DC: Woodrow Wilson International Center for Scholars.

McCarthy, J., I. Inkielewicz-Stępniak, J. J. Corbalan, and M. W. Radomski. 2012. Mechanisms of toxicity of amorphous silica nanoparticles on human lung submucosal cells *in vitro*: Protective effects of fisetin. *Chemical Research in Toxicology* 25:2227–35. doi:10.1021/tx3002884.

Meyer, D., and P. L. Williams. 2014. Toxicity testing of neurotoxic pesticides in *Caenorhabditis elegans*. *Journal of Toxicology and Environmental Health, Part B* 17:284–306. doi:10.1080/10937404.2014.933722.

Montazer, M., and M. Maali Amiri. 2014. ZnO nano reactor on textiles and polymers: Ex situ and in situ synthesis, application, and characterization. *Journal of Physical Chemistry. B* 118:1453–70. doi:10.1021/jp408532r.

Mu, Q., C. A. David, J. Galceran, C. Rey-Castro, L. Krzemiński, R. Wallace, F. Bamiduro, S. J. Milne, N. S. Hondow, R. Brydson, G. Vizcay-Barrena, M. N. Routledge, L. J. Jeuken, and A. P. Brown. 2014. Systematic investigation of the physicochemical factors that contribute to the toxicity of ZnO nanoparticles. *Chemical Research in Toxicology* 27:558–67. doi:10.1021/tx4004243.

Murray, C. B., C. R. Kagan, and M. G. Bawendi. 2000. Synthesis and characterization of monodisperse nanocrystals and close-packed nanocrystal assemblies. *Annual Reviews Materials Sciences* 30:545–610. doi:10.1146/annurev.matsci.30.1.545.

Murthy, S. K. 2007. Nanoparticles in modern medicine: State of the art and future challenges. *International Journal of Nanomedicine* 2:129–41.

Nanotech Project., 2014. Project on emerging nanotechnologies. Consumer Products Inventory. http://www.nanotechproject.org/cpi.

Nations, S., M. Long, M. Wages, J. D. Maul, C. W. Theodorakis, and G. P. Cobb. 2015. Subchronic and chronic developmental effects of copper oxide (CuO) nanoparticles on *Xenopus laevis*. *Chemosphere* 135:166–74. doi:10.1016/j.chemosphere.2015.03.078.

Oberdörster, G., V. Castranova, B. Asgharian, and P. Sayre. 2015. Inhalation exposure to carbon nanotubes (CNT) and carbon nanofibers (CNF): Methodology and dosimetry. *Journal of Toxicology and Environmental Health, Part B* 18:121–212. doi:10.1080/10937404.2015.1051611.

Oesch, F., and R. Landsiedel. 2012. Genotoxicity investigations on nanomaterials. *Archives of Toxicology* 86:985–94. doi:10.1007/s00204-012-0838-y.

Ong, C., L. Y. L. Yung, Y. Cai, B. H. Bay, and G. H. Baeg. 2015. *Drosophila melanogaster* as a model organism to study nanotoxicity. *Nanotoxicology* 9:396–403. doi:10.3109/17435390.2014.940405.

Panacek, A., R. Prucek, D. Safarova, M. Dittrich, J. Richtrova, K. Benickova, R. Zboril, and L. Kvitek. 2011. Acute and chronic toxicity effects of silver nanoparticles (NPs) on *Drosophila melanogaster*. *Environmental Science & Technology* 45:4974–79. doi:10.1021/es104216b.

Pandey, A., S. Chandra, L. K. S. Chauhan, G. Narayan, and D. K. Chowdhuri. 2013. Cellular internalization and stress response of ingested amorphous silica nanoparticles in the midgut of *Drosophila melanogaster*. *Biochimica et Biophysica Acta (BBA) - General Subjects* 1830:2256–66. doi:10.1016/j.bbagen.2012.10.001.

Park, J., S. Kim, J. Yoo, J.-S. Lee, J.-W. Park, and J. Jung. 2014. Effect of salinity on acute copper and zinc toxicity to *Tigriopus japonicus*: The difference between metal ions and nanoparticles. *Marine Pollution Bulletin* 85:526–31. doi:10.1016/j.marpolbul.2014.04.038.

Park, K. 2013. Toxicokinetic differences and toxicities of silver nanoparticles and silver ions in rats after single oral administration. *Journal of Toxicology and Environmental Health, Part A* 76:1246–60. doi:10.1080/15287394.2013.849635.

Park, M. V., A. M. Neigh, J. P. Vermeulen, L. J. De La Fonteyne, H. W. Verharen, J. J. Briedé, H. Van Loveren, and W. H. De Jong. 2011. The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. *Biomaterials* 32:9810–17. doi:10.1016/j.biomaterials.2011.08.085.

Philbrook, N. A., V. K. Walker, A. N. Afrooz, N. B. Saleh, and L. M. Winn. 2011b. Investigating the effects of functionalized carbon nanotubes on reproduction and development in *Drosophila melanogaster* and CD-1 mice. *Reproductive Toxicology* 32:442–48. doi:10.1016/j.reprotox.2011.09.002.

Philbrook, N. A., L. M. Winn, A. N. Afrooz, N. B. Saleh, and V. K. Walker. 2011a. The effect of TiO_2 and Ag nanoparticles on reproduction and development of *Drosophila melanogaster* and CD-1 mice. *Toxicology and Applied Pharmacology* 257:429–36. doi:10.1016/j.taap.2011.09.027.

Plantié, E., M. Migocka-Patrzałek, M. Daczewska, and K. Jagla. 2015. Model organisms in the fight against muscular dystrophy: Lessons from *Drosophila* and zebrafish. *Molecules* 20:6237–53. doi:10.3390/molecules20046237.

Poland, C. A., R. Duffin, I. Kinloch, A. Maynard, W. A. Wallace, A. Seaton, V. Stone, S. Brown, W. Macnee, and K. Donaldson. 2008. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nature Nanotechnology* 3:423–28. doi:10.1038/nnano.2008.111.

Pompa, P. P., G. Vecchio, A. Galeone, V. Brunetti, S. Sabella, G. Maiorano, A. Falqui, G.,. Bertoni, and R. Cingolani. 2011. *In vivo* toxicity assessment of gold nanoparticles in *Drosophila melanogaster*. *Nano Research* 4:405–13. doi:10.1007/s12274-011-0095-z.

Posgai, R., M. Ahamed, S. M. Hussain, J. J. Rowe, and M. G. Nielsen. 2009. Inhalation method for delivery of nanoparticles to the *Drosophila* respiratory system for toxicity testing. *Science of the Total Environment* 408:439–43. doi:10.1016/j.scitotenv.2009.10.008.

Posgai, R., C. B. Cipolla-McCulloch, K. R. Murphy, S. M. Hussain, J. J. Rowe, and M. G. Nielsen. 2011. Differential toxicity of silver and titanium dioxide nanoparticles on *Drosophila melanogaster* development, reproductive effort, and viability: Size, coatings and antioxidants matter. *Chemosphere* 85:34–42. doi:10.1016/j.chemosphere.2011.06.040.

Pulskamp, K., S. Diabaté, and H. F. Krug. 2007. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. *Toxicology Letters* 168:58–74. doi:10.1016/j.toxlet.2006.11.001.

Rajh, T., N. M. Dimitrijevic, M. Bissonnette, T. Koritarov, and V. Konda. 2014. Titanium dioxide in the service of the biomedical revolution. *Chemical Reviews* 114:10177–216. doi:10.1021/cr500029g.

Rajiv, S., J. Jerobin, V. Saranya, M. Nainawat, A. Sharma, P. Makwana, C. Gayathri, L. Bharath, M. Singh, M. Kumar, A. Mukherjee, and N. Chandrasekaran. 2016. Comparative cytotoxicity and genotoxicity of cobalt(II, III) oxide, iron (III) oxide, silicon dioxide, and aluminum oxide nanoparticles on human lymphocytes *in vitro*. *Human & Experimental Toxicology* 35:170–83. doi:10.1177/0960327115579208.

Ramel, C., and J. Magnusson. 1992. Modulation of genotoxicity in *Drosophila*. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 267:221–27. doi:10.1016/0027-5107(92)90066-B.

Rand, M. D., S. L. Montgomery, L. Prince, and D. Vorojeikina. 2014. Developmental toxicity assays using the *Drosophila* model. *Current Protocols Toxicology* 59:1.12.1-1.12.20.

Ranjan, S., M. K. Jayakumar, and Y. Zhang. 2015. Luminescent lanthanide nanomaterials: An emerging tool for theranostic applications. *Nanomedicine (London)* 10:1477–91. doi:10.2217/nnm.14.229.

Reis Éde, M., A. A. Rezende, D. V. Santos, P. F. Oliveria, H. D. Nicolella, D. C. Tavares, A. C. Silva, N. O. Dantas, and M. A. Spanó. 2015. Assessment of the genotoxic potential of two zinc oxide sources (amorphous and nanoparticles) using the i*n vitro* micronucleus test and the *in vivo* wing somatic mutation and recombination test. *Food and Chemical Toxicology* 84:55–63. doi:10.1016/j.fct.2015.07.008.

Ribeiro, D. A., V. Carlin, A. C. C. Fracalossi, and L. M. Oyama. 2009. Radiopacifiers do not induce genetic damage in murine fibroblasts: An in vitro study. *International Endodontic Journal* 42:987–91. doi:10.1111/iej.2009.42.issue-11.

Rubilar, O., M. Rai, G. Tortella, M. C. Diez, A. B. Seabra, and N. Durán. 2013. Biogenic nanoparticles: Copper, copper oxides, copper sulphides, complex copper nanostructures and their applications. *Biotechnology Letters* 35:1365–75. doi:10.1007/s10529-013-1239-x.

Rubio, L., B. Annangi, L. Vila, A. Hernández, and R. Marcos. 2016. Antioxidant and anti-genotoxic properties of cerium oxide nanoparticles in a pulmonary-like cell system. *Archives of Toxicology* 90:269–78. doi:10.1007/s00204-015-1468-y.

Salata, O. V. 2004. Applications of nanoparticles in biology and medicine. *Journal Nanobiotechnol* 2:3. doi:10.1186/1477-3155-2-3.

Santo, N., U. Fascio, F. Torres, N. Guazzoni, P. Tremolada, R. Bettinetti, P. Mantecca, and R. Bacchetta. 2014. Toxic effects and ultrastructural damages to *Daphnia magna* of two differently sized ZnO nanoparticles: Does size matter? *Water Research* 53:339–50. doi:10.1016/j.watres.2014.01.036.

Saptarshi, S. R., A. Duschl, and A. L. Lopata. 2015. Biological reactivity of zinc oxide nanoparticles with mammalian test systems: An overview. *Nanomedicine (London)* 10:2075–92. doi:10.2217/nnm.15.44.

Sato, T., T. Katakura, S. Yin, T. Fujimoto, and S. Yabe. 2004. Synthesis and UV shielding properties of calcia-doped ceria nanoparticles coated with amorphous silica. *Solid State Ionics* 172:377–82. doi:10.1016/j.ssi.2004.02.057.

Sato, Y., A. Yokoyama, K. I. Shibata, Y. Akimoto, S. I. Ogino, Y. Nodasaka, T. Kohgo, K. Tamura, T. Akasaka, M. Uo, K. Motomiya, B. Jeyadevan, M. Ishiguro, R. Hatakeyama, F. Watari, and K. Tohji. 2005. Influence of length on cytotoxicity of multi-walled carbon nanotubes against human acute monocytic leukemia cell line THP-1 *in vitro* and subcutaneous tissue of rats *in vivo*. *Molecular Biosystems* 1:176–82. doi:10.1039/b502429c.

Sharma, V., D. Anderson, and A. Dhawan. 2012. Zinc oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria mediated apoptosis in human liver cells (HepG2). *Apoptosis* 17:852–70. doi:10.1007/s10495-012-0705-6.

Shin, S. C., S. H. Kim, H. You, B. Kim, A. C. Kim, K. A. Lee, J. H. Yoon, J. H. Ryu, and W. J. Lee. 2011. *Drosophila* microbiome modulates host developmental and metabolic homeostasis via insulin signaling. *Science* 334:670–74. doi:10.1126/science.1212782.

Shvedova, A. A., V. Castranova, E. R. Kisin, D. Schwegler-Berry, A. R. Murray, V. Z. Gandelsman, A. Maynard, and P. Baron. 2003. Exposure to carbon nanotube material: Assessment of nanotube cytotoxicity using human keratinocyte cells. *Journal of Toxicology and Environmental Health, Part A* 66:1909–26. doi:10.1080/713853956.

Siddique, Y. H., A. Fatima, S. Jyoti, F. Naz, W. Khan, B. R. Singh, and A. H. Naqvi. 2013. Evaluation of the toxic potential of graphene copper nanocomposite (GCNC) in the third instar larvae of transgenic *Drosophila melanogaster* (hsp70-lacZ) Bg9. *PloS ONE* 8:e80944. doi:10.1371/journal.pone.0080944.

Siddique, Y. H., M. Haidari, W. Khan, A. Fatima, S. Jyoti, S. Khanam, F. Naz, A. Rahul, F. Singh, B. R. Beg, T. Mohibullah, and A. H. Naqvi. 2015. Toxic potential of copper-doped ZnO nanoparticles in *Drosophila melanogaster* (Oregon R). *Toxicology Mechanisms and Methods* 25:425–32. doi:10.3109/15376516.2015.1045653.

Siddique, Y. H., W. Khan, S. Khanam, S. Jyoti, F. Naz, B. R. Singh, and A. H. Naqvi. 2014. Toxic potential of synthesized graphene zinc oxide nanocomposite in the third instar larvae of transgenic *Drosophila melanogaster* (hsp70-lacZ) Bg 9. *BioMed Research International* 2014:1–10. doi:10.1155/2014/382124.

Siegrist, M., A. Wiek, A. Helland, and H. Kastenholz. 2007. Risks and nanotechnology: The public is more concerned than experts and industry. *Nature Nanotechnology* 2:67. doi:10.1038/nnano.2007.10.

Silva, R. M., C. Teesy, L. Franzi, A. Weir, P. Westerhoff, J. E. Evans, and K. E. Pinkerton. 2013. Biological response to nano-scale titanium dioxide (TiO_2): Role of particle dose, shape, and retention. *Journal of Toxicology and Environmental Health, Part A* 76:953–72. doi:10.1080/15287394.2013.826567.

Snyder-Talkington, B. N., Y. Qian, V. Castranova, and N. L. Guo. 2012. New perspectives *for in vitro* risk assessment of multiwalled carbon nanotubes: Application of coculture and bioinformatics. *Journal of Toxicology and Environmental Health, Part B* 15:468–92. doi:10.1080/10937404.2012.736856.

Spence, K. D. 1991. Structure and physiology of the peritrophic membrane. In *Physiology of the insect epidermis*, editors K. Binnington, and A. Retnakaran, 77–93. Victoria, Australia: CSIRO Publications.

Stocker, H., and P. Gallant. 2008. Getting started: An overview on raising and handling *Drosophila*. *Methods in Molecular Biology* 420:27–44.

Strawn, E. T., C. A. Cohen, and B. A. Rzigalinski. 2006. Cerium oxide nanoparticles increase lifespan and protect against free radical-mediated toxicity. *FASEB Journal* 20: A1356.

Su, Y., Y. He, H. Lu, L. Sai, Q. Li, W. Li, L. Wang, P. Shen, Q. Huang, and C. Fan. 2009. The cytotoxicity of cadmium based, aqueous phase–synthesized, quantum dots and its modulation by surface coating. *Biomaterials* 30:19–25. doi:10.1016/j.biomaterials.2008.09.029.

Su, Y., M. Hu, C. Fan, Y. He, Q. Li, W. Li, L.-H. Wang, P. Shen, and Q. Huang. 2010. The cytotoxicity of CdTe quantum dots and the relative contributions from released cadmium ions and nanoparticle properties. *Biomaterials* 31:4829–34. doi:10.1016/j.biomaterials.2010.02.074.

Tellam, R. L. 1996. The peritrophic matrix. In *Biology of the insect midgut*, 86–114. Dordrecht, The Netherlands: Springer.

Thill, A., O. Zeyons, O. Spalla, F. Chauvat, J. Rose, M. Auffan, and A. M. Flank. 2006. Cytotoxicity of CeO_2 nanoparticles for *Escherichia coli*, physicochemical insight of the cytotoxicity mechanism. *Environmental Science & Technology* 40:6151–56. doi:10.1021/es060999b.

Tian, H., H.-J. Eom, S. Moon, J. Lee, J. Choi, and Y. D. Chung. 2013. Development of biomarker for detecting silver nanoparticles exposure using a GAL4 enhancer trap screening in *Drosophila*. *Environmental Toxicology and Pharmacology* 36:548–56. doi:10.1016/j.etap.2013.05.013.

Tsyusko, O. V., S. S. Hardas, W. A. Shoults-Wilson, C. P. Starnes, G. Joice, D. A. Butterfield, and J. M. Unrine. 2012. Short-term molecular-level effects of silver nanoparticle exposure on the earthworm, *Eisenia fetida*. *Environmental Pollution* 171:249–55. doi:10.1016/j.envpol.2012.08.003.

Uboldi, C., G. Giudetti, F. Broggi, D. Gilliland, J. Ponti, and F. Rossi. 2012. Amorphous silica nanoparticles do not induce cytotoxicity, cell transformation or genotoxicity in Balb/3T3 mouse fibroblasts. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 745:11–20. doi:10.1016/j.mrgentox.2011.10.010.

Vales, G., E. Demir, B. Kaya, A. Creus, and R. Marcos. 2013. Genotoxicity of cobalt nanoparticles and ions in *Drosophila*. *Nanotoxicology* 7:462–68. doi:10.3109/17435390.2012.689882.

Vales, G., L. Rubio, and R. Marcos. 2016. Genotoxic and cell-transformation effects of multi-walled carbon nanotubes (MWCNT) following *in vitro* sub-chronic exposures. *Journal of Hazardous Materials* 306:193–202. doi:10.1016/j.jhazmat.2015.12.021.

Valizadeh, A., H. Mikaeili, M. Samiei, S. M. Farkhani, N. Zarghami, M. Kouhi, A. Akbarzadeh, and S. Davaran. 2012. Quantum dots: Synthesis, bioapplications, and toxicity. *Nanoscale Research Letters* 7:480. doi:10.1186/1556-276X-7-480.

Vecchio, G. 2015. A fruit fly in the nanoworld: Once again *Drosophila* contributes to environment and human health. *Nanotoxicology* 9:135–37. doi:10.3109/17435390.2014.911985.

Vecchio, G., A. Galeone, V. Brunetti, G. Maiorano, L. Rizzello, S. Sabella, R. Cingolani, and P. P. Pompa. 2012a. Mutagenic effects of gold nanoparticles induce aberrant phenotypes in *Drosophila melanogaster*. *Nanomedicine* 8:1–7.

Vecchio, G., A. Galeone, V. Brunetti, G. Maiorano, S. Sabella, R. Cingolani, and P. P. Pompa. 2012b. Concentration-dependent, size-independent toxicity of citrate capped AuNPs in *Drosophila melanogaster*. *PLoS ONE* 7:e29980. doi:10.1371/journal.pone.0029980.

Vega-Alvarez, S., A. Herrera, C. Rinaldi, and F. A. Carrero-Martínez. 2014. Tissue-specific direct microtransfer of nanomaterials into *Drosophila embryos* as a versatile *in vivo* test bed for nanomaterial toxicity assessment. *International Journal of Nanomedicine* 9:2031–41.

Venken, K. J., A. Sarrion-Perdigones, P. J. Vandeventer, N. S. Abel, A. E. Christiansen, and K. L. Hoffman. 2016. Genome engineering: *Drosophila melanogaster* and beyond. *Wiley Interdisciplinary Reviews: Developmental Biology* 5:233–67. doi:10.1002/wdev.2016.5.issue-2.

Völker, C., C. Boedicker, J. Daubenthaler, M. Oetken, and J. Oehlmann. 2013. Comparative toxicity assessment of nanosilver on three *Daphnia* species in acute, chronic and multi-generation experiments. *Plos One* 8:e75026. doi:10.1371/journal.pone.0075026.

Walkey, C., S. Das, S. Seal, J. Erlichman, K. Heckman, L. Ghibelli, E. Traversa, J. F. McGinnis, and W. T. Self. 2015. Catalytic properties and biomedical applications of cerium

oxide nanoparticles. *Environment Sciences Nano* 2:33–53. doi:10.1039/C4EN00138A.

Walters, C. R., P. Cheng, E. Pool, and V. Somerset. 2016. Effect of temperature on oxidative stress parameters and enzyme activity in tissues of Cape River crab (*Potamanautes perlatus*) following exposure to silver nanoparticles (AgNP). *Journal of Toxicology and Environmental Health, Part A* 79:61–70. doi:10.1080/15287394.2015.1106357.

Wang, B., N. Chen, Y. Wei, J. Li, L. Sun, J. Wu, Q. Huang, C. Liu, C. Fan, and H. Song. 2012a. Akt signaling-associated metabolic effects of dietary gold nanoparticles in *Drosophila*. *Scientific Reports* 2:563. doi:10.1038/srep00563.

Wang, T., X. Long, Y. Cheng, Z. Liu, and S. Yan. 2015. A comparison effect of copper nanoparticles versus copper sulphate on juvenile *Epinephelus coioides*: Growth parameters, digestive enzymes, body composition, and histology as biomarkers. *International Journal of Genomics* 2015:783021.

Wang, Z., N. Li, J. Zhao, J. C. White, P. Qu, and B. Xing. 2012b. CuO nanoparticle interaction with human epithelial cells: Cellular uptake, location, export, and genotoxicity. *Chemical Research in Toxicology* 25:1512–21. doi:10.1021/tx3002093.

Wason, M. S., and J. Zhao. 2013. Cerium oxide nanoparticles: Potential applications for cancer and other diseases. *American Journal Translat Researcher* 5:126–31.

Willhite, C. C., N. A. Karyakina, R. A. Yokel, N. Yenugadhati, T. M. Wisniewski, I. M. Arnold, F. Momoli, and D. Krewski. 2014. Systematic review of potential health risks posed by pharmaceutical, occupational and consumer exposures to metallic and nanoscale aluminum, aluminum oxides, aluminum hydroxide and its soluble salts. *Critical Reviews in Toxicology* 44 (Suppl sup4):1–8. doi:10.3109/10408444.2014.934439.

Wolf, M. J., H. Amrein, J. A. Izatt, M. A. Choma, M. C. Reedy, and H. A. Rockman. 2006. From The Cover: *Drosophila* as a model for the identification of genes causing adult human heart disease. *Proceedings of the National Academy of Sciences* 103:1394–99. doi:10.1073/pnas.0507359103.

Yadav, J. S., M. P. Lavanya, P. P. Das, I. Bag, A. Krishnan, R. Leary, A. Bagchi, B. Jagannadh, D. K. Mohapatra, M. P. Bhadra, and U. Bhadra. 2010. 4-*N*-Pyridin-2-yl-benzamide nanotubes compatible with mouse stem cell and oral delivery in *Drosophila*. *Nanotechnology* 21:155102. doi:10.1088/0957-4484/21/15/155102.

Yohan, D., and B. D. Chithrani. 2014. Applications of nanoparticles in nanomedicine. *Journal of Biomedical Nanotechnology* 10:2371–92. doi:10.1166/jbn.2014.2015.

Zhang, T., L. Wang, Q. Chen, and C. Chen. 2014a. Cytotoxic potential of silver nanoparticles. *Yonsei Medical Journal* 55:283–91. doi:10.3349/ymj.2014.55.2.283. Q10

Zhang, W.-D., L.-C. Jiang, Y.-X. Yu, and X.-L. Wei. 2014b. Electrodeposition of polyaniline onto TiO_2 nanoparticles/multiwalled carbon nanotubes for visible light photoelectrocatalysis. *Journal of Nanoscience and Nanotechnology* 14:7032–37. doi:10.1166/jnn.2014.8980.

Zhao, J., and V. Castranova. 2011. Toxicology of nanomaterials used in nanomedicine. *Journal of Toxicology and Environmental Health, Part B* 14:593–632. doi:10.1080/10937404.2011.615113.

Zhao, M.-X., and E.-Z. Zeng. 2015. Application of functional quantum dot nanoparticles as fluorescence probes in cell labeling and tumor diagnostic imaging. *Nanoscale Research Letters* 10:171. doi:10.1186/s11671-015-0873-8.