High-throughput COPAS assay for screening of developmental and reproductive toxicity of nanoparticles using the nematode Caenorhabditis elegans

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Abstract
With the rapid advancement and numerous applications of engineered nanomaterials (ENMs) in science and technology, their effects on animal health, environment and safety should be considered carefully. However, quick assessment of their effects on developmental and reproductive health and an understanding of how they cause such adverse toxic effects remain challenging, because of the fast-growing number of ENMs and the limitations of the different toxicity assays currently in use as well as lack of suitable animal model systems. In this study, we performed a high-throughput complex object parametric analyzer and sorter (COPAS) assay for assessing the developmental and reproductive toxicity of ENMs using Caenorhabditis elegans and provide descriptions of the data and their subsequent analysis. The results showed significant reproductive and developmental toxicity potential of different ENMs. We assessed the usefulness of this method in terms of error-free data, user-friendliness and results being consistent with those of visual, molecular and cellular studies. Moreover, the COPAS Biosort system could be used on a larger scale to screen thousands of chemicals, drugs, pharmaceuticals and ENMs. This study also indicates that the COPAS-based high-throughput screening system is highly reliable for the assessment of toxicity and health risks of NMs.

KEYWORDS
C. elegans, developmental toxicity, engineered nanomaterials, high-throughput screening, reproductive toxicity

1 | INTRODUCTION

The booming nanotechnology industry has raised concerns among the public about the environmental health and safety of engineered nanomaterials (ENMs). Worldwide research on ENMs and their commercial success have led to a rapid increase in the number of applications; however, limited understanding of aspects related to the environmental health and safety of ENMs remains a major obstacle for the future progress of nanotechnology (Qu, Brame, Li, & Alvarez, 2013). Currently, a key challenge is the very limited and often conflicting data available in the published literature and the fact that different ENMs are physicochemically heterogeneous makes it difficult to generalize the health risks associated with exposure to ENMs (Brohi et al., 2017). Therefore, there is an urgent need to have a clear understanding of the toxic effects of ENMs and to elucidate the mechanisms involved in their toxicity. In this regard, high-throughput screening (HTS) techniques aimed at accurately predicting and assessing the toxicity of ENMs are definitely and urgently needed.

With the growing numbers of ENMs, there is a huge demand from the scientific community as well as from legislative institutions for...
methods to test the safety of ENMs accurately and rapidly. HTS is considered a leading paradigm for tackling this challenge (Damoiseaux et al., 2011). It allows the examination of a large number of samples, concentrations, materials and experimental variations in a time- and cost-effective manner (Collins et al., 2017). Several HTS methods have been used for testing of ENM toxicity (Barrick, Châtel, Bruneau, & Mouneyrac, 2017; Jung et al., 2015; Nel et al., 2013). One of the better solutions for HTS of large numbers of ENMs is the complex object parametric analyzer and sorter (COPAS) Biosort system (Union Biometrica) (Pulak, 2006). The COPAS Biosort facilitates the rapid, accurate and efficient analysis, dispensing and/or sorting of a large number of nematodes by measuring their axial length (time of flight, TOF) as well as their size and internal structure (extinction, EXT).

In recent years, reproductive and developmental toxicity has increasingly been recognized as an important component of the overall toxicity of ENMs. In fact, several reports show that nanoparticles (NPs) can pass through biological membranes. This has been a cause of great concern with regard to the possible reproductive and developmental toxicity of ENMs (Brooking, Davis, & Illum, 2001; Ema, Kobayashi, Naya, Hanai, & Nakanishi, 2010; Williams, 2018). In this regard, Caenorhabditis elegans, a popular model organism for reproductive and developmental biology research is now being recognized as an attractive invertebrate model for high-throughput toxicological studies (Hunt, 2017). C. elegans growth assay is currently being used to screen the ToxCast Phase I and II libraries of the US Environmental Protection Agency (EPA), which comprise 1011 chemicals (Judson et al., 2010; Knudsen et al., 2009). In addition, the COPAS-based C. elegans HTS assay has been used in the screening of neurotoxicants (Boyd, Smith, Kissling, & Freedman, 2010) and for assessing the reproductive toxicity of drugs and environmental chemicals (Maurer, Ryde, Yang, & Meyer, 2015), effects of environmental toxicants on germline cells (Shin, Cuenca, Karthikraj, Kannan, & Colaiácovo, 2019), larval growth and development (Boyd et al., 2010), and chemical disruption of germline function (Allard, Kleinstreuer, Knudsen, & Colaiácovo, 2013). It has also been used for studying the mitochondrial function and morphology (Daniele et al., 2017) and developmental toxicity in axenic liquid cultures of C. elegans (Sprando, Olejnık, Cinar, & Ferguson, 2009), zebrafish, rats and rabbits (Boyd et al., 2016). C. elegans is also widely used in nanotoxicity assessment because it has many advantages that are useful for assessing nanotoxicity, such as transparent body and short reproductive life cycle (Choi et al., 2014; Wang, 2018; Wu, Xu, Liang, & Tang, 2019). However, reports on the reproductive and developmental toxicity potential of ENMs have been scarce and inconclusive. Therefore, in the present study, we performed COPAS Biosort HTS for the reproductive and developmental toxicity of selected ENMs in C. elegans.

2 | MATERIALS AND METHODS

2.1 | Maintenance of C. elegans

C. elegans strains were cultured at 20°C on nematode growth medium plates seeded with Escherichia coli strain OP50, as previously described (Brenner, 1974). The N2 Bristol strain was used as the wild-type strain, obtained from the Caenorhabditis Genetics Center (CGC). We synchronized a number of gravid adult C. elegans with 10% hypochlorite solution to isolate the eggs (Stiernagle, 2006), which were used in all the experiments.

2.2 | Selection and preparation of chemicals

To predict the toxic level in the screening assay using C. elegans by comparing with mammalian in vivo data associated with reproduction present in the Toxicological Reference Database (ToxRefDB, http://www.epa.gov/ncct/toxrefdb), we selected 16 chemicals for which in vivo multigenerational reproductive toxicity data are available. Descriptions of the selected chemicals and their mammalian in vivo endpoint data are presented in Table 1. The chemicals were divided based on their lowest-observed-adverse-effect level in a multigenerational study (MGLEL) (Martin, Judson, Reif, Kavlock, & Dix, 2009). Eight chemicals with one or more endpoints in MG-LEL were defined as positive reproductive toxicants, and eight chemicals that had no MG-LEL endpoints ≤500 mg/kg/day were considered as negative controls. All the chemicals were purchased from Sigma-Aldrich (Korea), and dissolved in dimethyl sulfoxide. The final concentrations of dimethyl sulfoxide and chemicals were 0.1% and 100 μM, respectively. Testing solution of chemicals was prepared in K-media (0.032 M KCl and 0.051 M NaCl) containing OP50 as food for C. elegans.

2.3 | Nanoparticles and physicochemical characterization

Seven types of commercially available NPs were used. These included the following: two types of amorphous silica NPs (aSiNPs), namely aSiNPs-189 (18.3 nm), which were obtained from the Nanoreg project (http://www.nanoreg.eu), and aSiNPs-116, which were purchased from Sigma-Aldrich; two types each of TiO2NPs (6 nm and 24.7 nm), CeO2NPs (33 nm) and AgNPs (16.7 nm), dispersed in deionized water containing 7% ammonium nitrate as a stabilizing agent and 8% emulsifiers, which were provided by Nanoreg; AgNPs (<150 nm), which were purchased from Sigma-Aldrich. The stock solutions were prepared in distilled water at a concentration of 2560 mg/L by sonication and were immediately used as test materials. K-medium was used as the exposure media for all NPs, except AgNPs (<150 nm), for which EPA moderately hard water (NaHCO3 96 mg/L, CaSO4·2H2O 60 mg/L, MgSO4 60 mg/L and KCl 4 mg/L) was used. NPs in the exposure media were characterized by photon dynamic light scattering (DLS, DLS-7000: Otsuka Electronics) and transmission electron microscopy (TEM; LIBRA 120; Carl Zeiss) as described previously (Chatterjee, Jeong, Yoon, Kim, & Choi, 2018). The exposure concentrations ranged from 0.0625 to 100 mg/L.
2.4 | High-throughput screening with the COPAS Biosort analysis

Development of C. elegans from L1 larvae to adult stage takes approximately 72 hours at 20°C. This time period was chosen for growth assays to allow for maximum development, while avoiding the production of offspring at later times. To determine the potential toxicity of the chemicals on larval development, 20 synchronized L1 nematodes were distributed by COPAS Biosort to each well of a 96-well plate. After 24, 48 and 96 hours of incubations, the sizes of worms in each well were recorded using COPAS Biosort by aspirating the exposure medium from the sample wells and measuring TOF, EXT and frequency for individual C. elegans. The sizes of nematodes were linked to specific C. elegans larval stages (Boyd et al., 2009). In the full reproduction assay, single young adult individuals were placed in a 96-well plate and incubated at 20°C for 72 hours. After the exposure, the number of offspring from one young adult were counted using COPAS Biosort. All samples were prepared with three replicates in a 96-well plate.

2.5 | Data and statistical analysis

To exclude measurements of objects other than nematodes (NPs aggregates), values were deleted if they deviated by one standard deviation at that EXT value from the TOF vs. EXT plots for an entire data set in all concentrations. The significance of differences among/between treatments was determined using one-way analysis of variance followed by post-hoc tests (Tukey, \( P < .05 \)) in SPSS 12.0KO (SPSS Inc.). We used MATLAB (R2017a) to carry out data plotting with \( P < .05 \) considered statistically significant.

3 | RESULTS

3.1 | Optimization of COPAS analysis

The COPAS Biosort EXT measurement reflects the amount of light absorbed as an object passes through the laser and has been successfully used as an indicator of size of C. elegans (Pulak, 2006). Before toxicity testing, we optimized the assessment of the developmental stage of C. elegans using COPAS-SELECT. Age-synchronized L1 larvae were monitored at 0, 24, 48 and 72 hours, by measuring EXT, TOF and frequency. To simplify the identification of the growth cycles of the nematode, a grid with log(EXT) and a histogram of all observations was plotted (Figure 1). Over the duration of the growth assay, the nematodes grew at an exponential rate. The growth in the control group varied from day-to-day and at different stages of nematodes over the 72-hour observation period.

### TABLE 1 List of negative and positive control chemicals with multigenerational reproductive toxicity data from the ToxRefDB

<table>
<thead>
<tr>
<th>CAS number</th>
<th>Chemical</th>
<th>Usage</th>
<th>Fertility</th>
<th>Implantations</th>
<th>Litter size</th>
<th>Ovary</th>
<th>Reproductive outcome</th>
<th>Reproductive performance</th>
<th>Viability postnatal day 4</th>
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<tr>
<td>101200-48-0</td>
<td>Tribenuron-methyl</td>
<td>Pesticide</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>34014-18-1</td>
<td>Tebuthiuron</td>
<td>Herbicide</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>87820-88-0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>51-03-6</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>181274-15-7</td>
<td>Propanocarbazonium</td>
<td>Herbicide</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>21087-64-9</td>
<td>Metribizin</td>
<td>Herbicide</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>23103-98-2</td>
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<td>Insecticide</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>22781-23-3</td>
<td>Bendiocarb</td>
<td>Insecticide</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>116714-46-6</td>
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<tr>
<td>741-58-2</td>
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<td>Herbicide</td>
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<td>68.2</td>
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<tr>
<td>12427-38-2</td>
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<tr>
<td>333-41-5</td>
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<td>Insecticide</td>
<td>35.2</td>
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<td>35.15</td>
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<td>2310-17-0</td>
<td>Phosalone</td>
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<td>Pesticide</td>
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<td>23.2</td>
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<tr>
<td>68694-11-1</td>
<td>Triflumizole</td>
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<td>60168-88-9</td>
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<td>0</td>
<td>1.2</td>
<td>2.5</td>
<td>0</td>
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</tr>
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</table>

NOAEL, no-observed-adverse-effect level; ToxRefDB, Toxicological Reference Database.
3.2 Validation of COPAS-based reproductive and developmental toxicity test in *C. elegans*

COPAS-based development and reproduction screening tests for *C. elegans* were validated using eight chemicals, which are verified for their reproductive and developmental toxicity potential in mammalian models, as positive controls, and eight chemicals with no such indications as negative controls (Figure 2). The chemicals in the positive control group included dicofol, bensulide, phosalone, fenarimol, diazinon, manebr, triflumizole and navaluron, and those in the negative control group included tebuthiuron, metribuzin, bendiocarb, tribenuron-methyl, piperonyl butoxide, tralkoxydim, propoxycarbazone sodium and pirimicarb. In the results of developmental tests for the negative control group, the log(EXT) value at which the peak appeared increased with time although it varied for different chemicals (Figure 2A). It was also found that the 72-hour data had a high frequency peak at low log(EXT) value and therefore the negative control had little effect on the early reproductive potential. On the other hand, in the results for the positive control group, it was difficult to identify the peak or the increase over time clearly (Figure 2B). In addition, unlike in the negative control group, no peak corresponding to L1 was observed at 72 hours,

**FIGURE 1** Growth of *Caenorhabditis elegans* from the L1 to adult stage. Untreated *C. elegans* were incubated at 20°C and sampled at 0, 24, 48 and 72 h. Histogram shows optical density (log(EXT)) versus frequency distributions. At 72 h, adult nematodes (high EXT) and their offspring (low EXT) were observed. EXT, extinction

**FIGURE 2** Observed frequency distributions of log(EXT) of *Caenorhabditis elegans* exposed to negative and positive control chemicals. For development and initial reproduction analysis, age-synchronized L1 larvae were exposed to each chemical for 96 h and EXT and frequency were measured every 24 h. A, Negative control group chemicals. B, Positive control group chemicals. EXT, extinction
indicating that the chemicals in the positive control group affected not only the development but also the early reproductive potential. To confirm the reproductive potential by measuring the number of offspring, age-synchronized young adults were exposed to each chemical for 72 hours (Figure 3). No reproductive toxicity was observed for any of the chemicals in the negative control group, and the number of offspring tended to be lower in the positive control group than in the negative control group. Severe reproductive toxicity was observed for dicofol, phosalone, diazinon and triflumizole. We validated the developmental and reproductive toxicity assay using COPAS Biosort, which directly counts the number of nematode offspring, and the number of larvae produced during this time period is an indication of the effect of the toxicant on nematode fecundity/reproductive potentials. Overall, our results indicate that the COPAS Biosort system is a good screening tool for the assessment of reproductive and developmental toxicity in mammals using C. elegans as a model system.

3.3 | Physicochemical characterization of engineered nanomaterials

The DLS and TEM analyses were performed for physicochemical characterization of ENMs in the C. elegans exposure media. The hydrodynamic diameters and electrophoretic mobility of ENMs are summarized in Table 2. The TEM images show that the sizes of the NMs in deionized water were close to their labeled monomeric sizes. In the tested media, the measured hydrodynamic diameter ranged from 126 to 379 nm, with the zeta potential ranging from -47.5 to 33 mV.

3.4 | Developmental and reproductive toxicity assays of engineered nanomaterials using COPAS analysis

We tested both developmental and early reproductive toxicity assays as HTS tools to estimate the potential toxicities of the ENMs using COPAS. The 72-hour developmental dynamics was first screened using AgNPs, SiO2NP, TiO2NPs and CeO2NPs in C. elegans (Figure 4). Results show that significant early reproductive toxicity was observed in the AgNP-treated group, whereas no reproductive toxicity was observed in the CeO2NP-, TiO2NP- and SiO2NP-treated groups. However, in the TiO2NP-treated group, an abnormally high peak was observed between EXT 2 and 3 at 72 hours, which seemed to be due to the presence of aggregated NPs that were aspirated along with the nematodes, and resulted in high frequency values. In this study, the optimized COPAS screening method for soluble compounds were applied for NPs. However, unlike for the chemicals in the positive and negative control groups, NPs are not soluble and were in dispersed condition in the culture media. In COPAS analysis, dispersed NPs in the media create interference with reproduction (frequency) data and this is considered as one of the limitations of COPAS for HTS of NPs.

3.5 | Assessment of dose- and size-specific response on the development of C. elegans by COPAS analysis

Using the COPAS analysis, we examined the developmental toxicity of four NMs at different concentrations. Developmental toxicity of NPs was observed at 48 hours for a broad range of exposure concentrations from 0.0625 to 100 mg/L (Figure 5). No developmental toxicity was observed for any of the four NMs up to a concentration of 0.5 mg/L. However, concentration-dependent toxicity was observed for AgNPs at concentrations higher than 1 mg/L. Similarly, SiO2NP- and TiO2NP-treated groups showed significant developmental toxicity at concentrations higher than 10 mg/L, whereas, 25 mg/L CeO2NPs caused toxicity in C. elegans.

Additionally, we studied whether the 48-hour TOF values for the developmental toxicity index could be applied to test the size-dependent toxicity of NPs by using different-sized NPs (16.7 and <150 nm for AgNPs; 16.2 and 200 nm for SiO2NPs; 6 and 24.7 nm...
for TiO$_2$NPs) (Figure 6). Distinct size-dependent toxicity was observed in nematodes treated with AgNPs and SiO$_2$NPs at all concentrations higher than 10 mg/L. However for TiO$_2$NPs, size-dependent toxicity was observed only at the highest concentration tested (i.e., 100 mg/L), which might be because the size difference of the two TiO$_2$NPs was not as evident as it was for AgNPs or SiO$_2$NPs. These results collectively suggest that the COPAS-based HTS assay could be applicable for testing the developmental toxicity of NPs, and the exposure concentration and size-dependent toxicity can be successfully assessed using this HTS method.

### 4 | DISCUSSION

NMs are being used extensively in a variety of applications at such a rate that cannot be matched by safety/toxicity assessments. There is an urgent need for consistent and accurate HTS systems for rapid toxicity assessment of emerging NMs (Ban, Zhou, Sun, Mu, & Hu, 2018). The present study showed that the HTS strategy using C. elegans can be successfully applied for toxicity assessment of ENMs, as has been reported in previous studies on NMs and various environmental toxicants (Hunt et al., 2014; Jung et al., 2015; Mashock et al., 2016; Shin et al., 2019). The developmental and reproductive toxicity assay described in this study used the COPAS Biosort assay for counting the number and measuring the size of C. elegans at L1 larval and adult stages. This period was chosen to coincide with the developmental stage when the number of germ cells reached its maximum, but before the embryonic membrane became impermeable (Epstein et al., 1995). Before testing the toxicity of ENMs, preliminary experiments focused on optimizing the method using the toxicity potential of different known reproductive toxicants. An exposure time of 48-72 hours was chosen to maintain a relatively low number of offspring. This exposure time avoided overcrowding in the wells and allowed nematode counts to remain within the sampling limits of the Biosort (Boyd, et al., 2010). Exposures to ENMs and toxicants were started at the L1 stage to maximize the chance of observing potential chemical effects on reproduction and development, while avoiding potential effects on the growth of offspring (Boyd, et al., 2010). Our results showed that C. elegans is an efficient animal model for HTS of the reproductive and developmental toxicities using COPAS analysis.

Among several HTS assays, the COPAS Biosorts represent recent advances in HTS analyses of C. elegans (Pulak, 2006). Before the invention of COPAS, library screening and toxicity assessment of compounds in model organisms used manual processes and were, thus, very slow, tedious and simply impractical as they could only be performed for one organism at a time under a microscope. The COPAS system can analyze about 1 million C. elegans individuals in 8 hours (Pulak, 2006). The screening time with the COPAS analysis consists of 48-72 hours of exposure and 45 minutes of reading for each 96-well plate. Because exposures can be performed simultaneously and each plate adds only an additional 45 minutes of reading time, a library of 1000 compounds can be screened in triplicate in only 4 days. We used COPAS for assessing the developmental and reproductive

TABLE 2

<table>
<thead>
<tr>
<th>Engineering Nanomaterials</th>
<th>Polymorph</th>
<th>Diameter (nm)</th>
<th>Transmission electron microscopic image</th>
<th>Zeta potential (mV)</th>
<th>Hydrodynamic diameter (nm)</th>
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</thead>
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<tr>
<td>AgNPs</td>
<td>Metallic</td>
<td>16.7</td>
<td><img src="#" alt="Image" /></td>
<td>-47.5</td>
<td>200.78 ± 4.69</td>
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<tr>
<td>aSiNP-116</td>
<td>Amorphous</td>
<td>20-50</td>
<td><img src="#" alt="Image" /></td>
<td>-10.02</td>
<td>333 ± 11.19</td>
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<tr>
<td>aSiNP-189</td>
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<td><img src="#" alt="Image" /></td>
<td>-47.5</td>
<td>200.78 ± 4.69</td>
</tr>
<tr>
<td>CeO$_2$NPs</td>
<td>Amorphous</td>
<td>-11 ± 3</td>
<td><img src="#" alt="Image" /></td>
<td>166.63 ± 287</td>
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<tr>
<td>TiO$_2$NPs</td>
<td>Metallic</td>
<td>1.5</td>
<td><img src="#" alt="Image" /></td>
<td>166.63 ± 287</td>
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</tr>
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</table>

N/A, not applicable; NPs, nanoparticles.
toxicity of AgNPs, SiO₂NPs, TiO₂NPs and CeO₂NPs, and our results prove that it is an efficient and reliable technology for fast screening of ENMs or other toxicants that alter the reproduction and growth of C. elegans. We focused on known reproductive toxicants to address the gap in our current ability to assess the potential toxicity of NPs. However, the assay described here is also applicable to other
chemicals and ENMs for the safety assessment of NPs and small molecule assays by analyzing the reproductive toxicity in animals.

In recent years, the incorporation of COPAS assays has been attempted in toxicological screening studies (Pulak, 2006). The results of these studies showed that COPAS is a suitable and reliable system for assaying the changes in mitochondrial morphology and activity (Daniele et al., 2017; Hunt, Olejnik, Bailey, Vaught, & Sprando, 2018), heavy metal toxicity (Hunt, Olejnik, & Sprando, 2012), toxicity assessment of neurotoxicants (Boyd, et al., 2010), and environmental toxicants (Boyd, McBride, Rice, Snyder, & Freedman, (2010), and enables the screening of drug libraries (Cho, Behnam Azad, Luyt, & Lewis, 2013), metal NMs (Jeong et al., 2018; Kim et al., 2017) and phenotype profiling of C. elegans in different chemical environments (Gao et al., 2018). However, COPAS has several limitations. One of the limitations of using COPAS for measurements in C. elegans is the possibility of extraneous materials, such as bacteria, discarded cuticles or chemical precipitates being aspirated along with the nematodes (Smith et al., 2009). Another limitation of the COPAS assay is its susceptibility to the effects of ambient temperature and humidity, and the introduction of artifacts from improper gating or handling (Collins et al., 2017; Hunt et al., 2012). The instrumentation for the COPAS assay is also very expensive, and is not widely available in many laboratories, which limits its use for toxicity testing with C. elegans (Fernandez et al., 2012). In addition, the COPAS system only provides a limited range of data on features such as length and width of worm, and fluorescence intensity. Besides these minor limitations, COPAS provides a low-cost, high-speed and strong predictive value, and fulfills the requirement for the first-pass assessment of toxicity. Furthermore, it offers insights into the mechanism of reproductive and developmental toxicity in animals.

With the increasing diversity of engineered NMs being considered for large-scale use, this COPAS HTS assay would facilitate rapid screening of the toxicity of NMs, allowing risk assessment of NPs in a timely manner. In addition, with the recent technical advances in C. elegans handling, culture and phenotyping, it is now increasingly possible to conduct mass screens in whole, intact organisms for developmental toxicity risk assessment assays. Therefore, the COPAS HTS assay using C. elegans can be applied at a larger scale for screening thousands of chemicals, drugs, pharmaceuticals and ENMs. However, our results clearly demonstrate several limitations of using COPAS HTS that would need to be technically improved in the future for better HTS assays. There is a need for modification of the COPAS assay with better computational tools, which will provide an opportunity for the creation of faster and error-free toxicity screening on a wider scale using C. elegans. It is our hope that, in future, COPAS technology will be widely used for ecotoxicity and health risk assessment of emerging pollutants, such as microplastics and nanoplastics.

CONFLICTS OF INTERESTS

The authors have no conflicts of interest to report.

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REFERENCES

nanoparticles. *Environmental Science and Technology*, 52(17), 9666–9676. https://doi.org/10.1021/acs.est.8b02757


