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Multivariate modeling of engineered nanomaterial features associated with developmental toxicity

Kimberly T. To^{1,2}, Lisa Truong^{5,6}, Sabrina Edwards⁵, Robert L. Tanguay^{5,6}, David M. Reif^{1,2,3,4}

¹Bioinformatics Research Center, North Carolina State University, Raleigh, NC, USA

²Bioinformatics Graduate Program, North Carolina State University, Raleigh, NC, USA

³Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA

⁴Center for Human Health and the Environment, North Carolina State University, Raleigh, NC, USA

⁵Sinnhuber Aquatic Research Laboratory, Oregon State University, Corvallis, OR, USA

⁶Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, USA

Abstract

Despite the increasing prevalence of engineered nanomaterials (ENMs) in consumer products, their toxicity profiles remain to be elucidated. ENM physicochemical characteristics (PCC) are known to influence ENM behavior, however the mechanisms of these effects have not been quantified. Further confounding the question of how the PCC influence behavior is the inclusion of structural and molecular descriptors in modeling schema that minimize the effects of PCC on the toxicological endpoints. In this work, we analyze ENM physico-chemical measurements that have not previously been studied within a developmental toxicity framework using an embryonic zebrafish model. In testing a panel of diverse ENMs to build a consensus model, we found nonlinear relationships between any singular PCC and bioactivity. By using a machine learning (ML) method to characterize the information content of combinatorial PCC sets, we found that concentration, surface area, shape, and polydispersity can accurately capture the developmental toxicity profile of ENMs with consideration to whole-organism effects.

Keywords

Engineered nanomaterials; Predictive modeling; Feature analysis; Developmental toxicity; zebrafish

1. Introduction

Nanoparticles are small materials defined by having size-dependent properties and at least one dimension measuring between 1 and 100nm. Nanoparticles can arise from naturally occurring processes, as byproducts of manufacturing, or in the case of engineered nanomaterials (ENMs), specifically designed to be on the nanoscale. Engineered nanomaterials are defined as exhibiting behavior unique to materials within its size range. The behavior of ENMs in biological systems is thought to be influenced in part by their size, but also other physicochemical characteristics (PCC), such as polydispersity or surface area (Duffin et al., 2007; Kim et al., 2013). These characteristics stand to improve existing consumer products in both quality and durability, as evidenced by a projected increase in nanotechnology product market value from \$34.3B in 2015 to \$90.5B in 2021 (McWilliams, 2017). In addition, the National Nanotechnology Initiative overseeing nanotechnology-related research and development from over 20 U.S. agencies has a reported 2019 budget of \$1.395 billion, indicating consistent interest in developing nanotechnology (NSET, 2005).

Because of the increasing interest in ENM implementations, the toxicity profiles of ENMs must be characterized to prevent potentially hazardous effects on the general population. Toxicity of ENMs has been observed in multiple animal models. For example, compared to control, decreased viability and transgenerational toxicity have been observed in *D. melanogaster* after ingestion of various ENMs (Anand et al., 2017; Raj et al., 2017), genotoxicity and increase in reactive oxygen species (ROS) in *C. elegans* (Gonzalez-Moragas et al., 2017; Kong et al., 2017; Rogers et al., 2015), and genotoxicity, developmental toxicity, and lung injury in rodents (Duke et al., 2017; Ho et al., 2017; Rahman et al., 2017; Wan et al., 2017; Y. Zhang et al., 2017). A challenge in developing toxicity profiles is that novel ENMs can be developed quickly; therefore, ENM toxicity profiling must include predictive models that can link ENM characteristics with their toxicity.

Development of an accurate predictive model depends on a large sample of toxicity data so that nuanced effects of particular ENM PCC can be adequately aligned to a toxicity endpoint. However, developing such a dataset is hampered by difficulties in merging existing datasets due to inconsistencies in reporting, data quality concerns, and disparity in reported ENM characteristics (Puzyn et al., 2018). Existing predictive models for ENM toxicity have used *in vitro* measurements of toxicity paired with ENM PCC and theoretical molecular descriptors. Linear regression methods have been previously described, with each models' results depending on the variable selection criteria. For example, analyses of cytotoxicity in *E. Coli* from exposure to 17 different metal oxides resulted in a model using only enthalpy of formation of a gaseous cation (H_{Me+}) (Puzyn et al., 2011), a model using only the charge of a metal cation corresponding to a given oxide (χ_{OX}) (Kar et al., 2014), and a two variable model using polarization force (Z/r) and H_{Me+} (Hu et al., 2009). It is likely that these differences are derived from not only the variable selection method, but correlations among the selected descriptors. It is therefore necessary to evaluate the ENM descriptors for their influence on a particular endpoint before attempting to select variables for predictive modeling. While there have been promising leads in the use of computationally derived ENM descriptors to model cytotoxicity in bacteria (Toropova et al., 2016), there have been

no attempts to use ENM PCC to model developmental toxicity. By identifying the PCC that contribute to developmental toxicity, we can calculate safe thresholds for PCCs to be used in nano-enabled products.

Here, we characterize combinations of PCC from a diverse panel of ENMs for their association with multivariate measures of developmental toxicity when tested in concentration-response in embryonic zebrafish. In modeling features associated with developmental consequences, we utilize manufacturer-derived PCC to characterize the relationship matrix amongst characteristics themselves. Mapping of PCC to toxicity endpoints have been studied in the context of linear models, yet we find complex patterns and feature-selection dependencies that confound traditional statistical methods. Therefore, we use a machine learning method that removes this dependency while capturing the intricate within-characteristic relationships corresponding to ENM toxicological behavior. Specifically, we take advantage of the machine learning method, random forests decision trees, which allows for stability in the predictions of our moderately-sized dataset. Due to the known non-monotonic dose-response observed in ENM studies (Larson et al., 2014; Powers et al., 2011a), the random forests procedure overcomes associated limitations by considering numeric equivalencies across our multi-concentration curves. Our model identifies concentration, surface area, shape, and polydispersion as influential characteristics associated with developmental toxicity.

2. Materials and methods

2.1. ENM Characterization

As a part of the National Institute of Environmental Health Sciences (NIEHS) - Nanomaterials Health Implications Research (NHIR) Consortium, a total of 15 ENMs (Table 1) were provided by the Engineered Nanomaterials Resource and Coordination Core and precisely synthesized and dispersed in ultrapure water using published methods (Cohen et al., 2018; DeLoid et al., 2017). The materials were nominated within the NHIR consortium to evaluate toxicity of metal, metal oxide, and metal sulfide ENMs. When uniformly dispersed, physico-chemical properties are measured and provided for all members of the consortium. The measurements are summarized in Table 2 and Figure S1.

2.2 Experimental Design

2.2.1. ENM Exposure Solutions—To gain confidence in established dispersion methods provided (DeLoid et al., 2017), and provided physico-chemical properties, the 15 ENMS were dispersed in ultrapure water, and the mean hydrodynamic size, polydispersity index, and zeta potential was measured in the suspension at 0 and 24 hours. These values were compared to the NHRI provided characterization reports. The measurements were within 15% difference. A master stock was made at 1000 µg/mL.

2.2.2. Zebrafish—For these studies, adult Tropical 5D zebrafish were raised at Oregon State University, Sinnhuber Aquatic Research Laboratory (SARL) in accordance with protocols approved by the OSU institution Animal Care and Use Committee (IACUC) under ACUP 5113. The zebrafish were housed in standard laboratory conditions (28°C with 14 h

light: 10 h dark photo cycle) and fed twice daily with appropriate sized Gemma Micro (Skretting Inc, Tooele, France), without any live food supplement (Barton et al., 2016). Zebrafish are housed in a density of 1000 per 100 gallon, and Adult care and reproductive techniques followed the Institutional Animal Care and Use Committee protocols at Oregon State University. For the developmental toxicity testing, embryos were collected and staged at 3 hours post fertilization. Embryos were dechorionated at 4 hours post fertilization (hpf) using an automated dechorionator (Mandrell et al., 2012) and pronase enzyme (83 μ L of 25.3 U/ μ L; Roche Indianapolis, IN, USA). At 6 hpf, dechorionated embryos were placed into individual wells of a 96-well plate prefilled with test solution made up with ultrapure water. A total of 6 concentrations were tested (0, 2.32, 5, 10.7, 23.2, and 50 μ g/mL) on two replicate plates, with $n=16$ per plate ($n=32$ total per concentration). The plates were sealed with parafilm sandwiched between the plate and lid, and wrapped in aluminum foil until 120 hpf. A total of 22 morphological endpoint data was collected at 24 (4 endpoints) and 120 hpf (18 endpoints) (Truong et al., 2011). The data is collected in a binary manner and stored in a laboratory information management system (Truong et al., 2014) For each concentration-ENM pair, we summarize the 18 endpoints using weighted Aggregate Entropy (wAggE) to characterize the severity of toxicity. Calculations for wAggE are detailed in (G. Zhang et al., 2017). Briefly, the endpoint vector is weighted according to the coefficients of a fitted logistic regression model for response probability and summed across replicates. The resulting variable, wAggE, is a continuous value where increasing values correlate to increasing toxicity (Table 3). We estimate the distribution of wAggE with a χ^2 -distribution with 3 degrees of freedom (Zhang et al., 2016) and define a toxicity threshold with the 50% quantile ($\chi^2_{0.5} = 2.37$). Thus, we obtain a summary measure of developmental toxicity, wAggE, calculated for each of 15 nanomaterials at 6 different nominal concentrations (0.00, 2.32, 5.00, 10.70, 23.20, 50.00 μ g/mL) with a range of (0,10.53).

2.3. Modeling

The full dataset contains 90 rows, each corresponding to a unique ENM-concentration pair and descriptor set. Each ENM-concentration pair has a calculated wAggE value derived from aggregation across 3,240 individual fish. Among the 13 PCC, total pore volume, average pore size, and density were missing for citrate-capped 15nm gold (Au) nanoparticles and citrate-capped 20nm silver (Ag) nanoparticles. Median Feret diameter, aspect ratio, circularity, and roundness were missing for silicon oxide (SiO₂) nanoparticles. These missing data were imputed by performing random forests imputation on the matrix of PCCs. Random forests imputation initializes with mean imputations and iteratively updates these values through a series of 100 sum-of-trees models (Stekhoven and Buhlmann, 2012).

The training data were fit to a random forests sum-of-trees model using 1,000 trees. Random forests decision trees are an ensemble learning method that fit decision trees to resampled datasets ($T = 1,000$) and combine the results of each tree for a final aggregated prediction (Breiman, 2001). For each split within each tree, $m=4$ randomly selected variables are considered for a splitting rule. Rows not included in the resampled dataset are predicted as “out-of-bag” samples. Random forests trees improve the stability of a single decision tree’s predictions by reducing variance. Variable importance can be assessed using percent increase in Mean Squared Error (“%IncMSE”), where the original tree results are compared

with results from a tree rebuilt with values permuted for any single variable. We first fit a “full” model using concentration and all 13 PCC as input variables, and the estimated variable importance is reported. We then fit a reduced random forests model using only the top 4 variables. We also fit a baseline model using only concentration. Out-of-bag Mean Squared Error (MSE) and predicted R^2 values were calculated for each model to assess and compare model fit. MSE is a unitless measure of how distant the predicted wAggE is from the observed wAggE, so that MSEs closer to 0 indicate better fit. Predicted R^2 measures how well the models fit the out-of-bag sample data, with values closer to 0 indicating poor fit and values closer to 1 indicating good fit. A Wilcoxon signed-rank test was performed to test the null hypothesis that the predicted wAggE and observed wAggE values are derived from the same distribution.

An external validation set of three silver nanoparticles (10, 20, 30nm) was used to assess the model performance. The silver nanoparticles were purchased from Nanocomposix (San Diego, CA) and stored in 4°C in the dark. The particles were part of their BioPure Silver Nanosphere (Bare-Citrate) that had sizes ranging from 10 to 110 nm (nominal). The characterization sheets can be observed in the SI. The samples were suspended in ultrapure and exposed as described above. An additional three-variable model was derived, consisting of reported features common between the validation set and the reduced four-variable model. The applicability domain of the model is defined by the range of values within the model, so that selection of the validation set materials ensured interpolation. All analyses were performed using the R Statistical Language (Team and R Development Core Team, 2016).

3. Results

3.1. Physicochemical characteristic analysis

Distributions of the characteristics are shown in Table 2. Notably, the ranges for the descriptors are varied, which would typically require some standardization of the input variables prior to modeling. The decision tree model overcomes these marked differences in PCC distributions by relying on split rules for predictive performance rather than direct relationships between the PCC and endpoints.

Correlations between the quantitative PCC (Figure S1) show generally low correlation among descriptors. As expected, roundness is highly correlated with both aspect ratio and circularity, demonstrating a group that collectively describes the shape. Similarly, the diameter is negatively correlated with total pore volume, since we expect fewer pores in smaller ENM due to the available surface area. Hydrodynamic diameter describes both general size and particle distribution in solution, evidenced by its (absolute) correlation with median diameter and zeta potential. Conductance shows high correlations with average pore size and hydrodynamic diameter. ENM morphology has been previously shown to affect electrical conductance of the ENM; specifically, the greater pore size may provide more surfaces for ion transfer (Park et al., n.d.). The interactions made possible by porosity would likely affect the ENM's ability to agglomerate, resulting in the observed correlation between conductance and hydrodynamic diameter. These correlation patterns indicate redundancy in the feature space, suggesting that subsets may effectively capture relevant information.

3.2. The full model identifies key physicochemical characteristics

The full model built with all 14 variables (concentration and 13 PCC) uses concentration for most splitting rules, followed by a marked break in split rule inclusion for the remaining features (Figure 1). The inclusion of each variable in each of 1,000 trees is relatively high, reinforcing the partial redundancy evident in our correlation matrix. However, this exchangeability is teased apart by the variable importance metric (%IncMSE) shown in Table 4. Circularity and polydispersity index (PDI) have the highest importance metrics (22.45 and 19.79, respectively), followed by surface area and concentration (16.44 and 16.14, respectively). This is consistent with existing studies that show that although size, and therefore surface area has a great influence on toxicity, additional descriptors modify the response to ENMs (Boonrungsiman et al., 2017; Gajewicz et al., 2017; Liu et al., 2014; Moon et al., 2019; Powers et al., 2011b)

Polydispersion (PDI) can describe particle agglomeration, and thus particle size distribution in media, indicating heterogeneity of agglomerate sizes within the solution. Evidence has shown that particle dispersion plays a role in toxicity in that less agglomerated solutions maintain the small size of ENMs, allowing for their continued passage through cellular membranes (Zook et al., 2011). In one study, the toxicity of silver nanoparticles (AgNPs) was observed to be the result of not only particle size or surface coating, but the test media which alters the ability of these materials to agglomerate (Kim et al., 2013).

Similarly, circularity describes shape and influences the measurements of roundness and aspect ratio. In this work, we considered three shape-based characteristics: aspect ratio, roundness, and circularity. Indeed, shape plays a critical role in ENM behavior by affecting cellular uptake and exocytosis (Oh and Park, 2014). Circularity was shown to affect exocytosis by hindering macrophage uptake and clearance (Bigdeli et al., 2015). In another instance, gold nanotriangles, nanorods, and nanostars were shown to follow differential endocytotic pathways in a shape-dependent manner (Xie et al., 2017).

We, therefore, find that these top three physicochemical characteristics capture the dynamic *in vivo* behavior of ENMs by capturing their potential to change in size and traverse through cellular systems.

3.3. Reduced random forests decision tree shows similar performance to full model

The top four variables according to variable importance were selected for a reduced 4-variable random forests model in order to assess the sufficiency of this feature subset for predicting toxicity. We also fit a single variable model using the experimental parameter, concentration as a baseline model for assessment of our models.

According to MSE and R^2 , the concentration-only model shows poor performance ($MSE_{Null} = 6.48$, $R^2 = -0.05$). The full model demonstrates higher accuracy ($MSE_{Full} = 2.37$; $R^2_{Full} = 0.62$). The 4-variable model using circularity, PDI, concentration, and surface area performed equivalently (Table 5) to the full model, highlighting how comprehensively the 3 physicochemical characteristics capture the overall toxic effect of the ENMs. The equivalencies in predictive performance indicate the ability of these descriptors to account

for the subtle effects of the remaining, less important variables on the toxicological profile of the ENMs.

3.4. Underprediction of more toxic ENMs improves with the inclusion of PCC

We want to keep underprediction minimal to maximize sensitivity for identifying toxic responses. We observe that across all models, wAggE is underpredicted for ENMs with higher toxicity ($wAggE > \chi^2_{0.5} = 2.37$) (Figure 2). The prediction metrics and the results of a Wilcoxon signed-rank test demonstrate that the general structure of the toxicity distribution is maintained with the full and four-variable models ($p_{Full} = 0.56$, $p_{4var} = 0.74$, $p_{1var} = 0.09$). The concentration-only model shows some consistency in slight overpredictions of toxicity for ENMs with lower toxicity ($wAggE < \chi^2_{0.5} = 2.37$), though ENM with higher wAggE measurements are drastically underpredicted. The four-variable model shows improvements to the concentration-only model. Although predictions for more toxic ENMs are still underpredicted, the lower MSE indicates the PCC influence on toxicological response.

If we consider each specific ENM, we find that the silver-based ENM and zinc oxide (ZnO) have the highest MSE across models due to the heightened toxic response that is underpredicted by the models. However, the overall model MSE is relatively low and the results of the Wilcoxon signed-rank test show that the full and four-variable model predictions can capture the global developmental toxicity profile for these ENM.

3.5. Variable wAggE responses across PCC reveal intricate characteristic interactions for toxicity response

Across each of these variables, we observe varying response patterns as variable values increase (Figure S2). The experimental variable, concentration, was used most frequently among split rules, so we expect some concentration-based pattern with wAggE. A 2014 review of ENM toxicity studies found evidence of 11 hormetic dose-response curves in 13 in vitro studies and 16 in vivo studies, showing a consistent, non-linear trend (Iavicoli et al., 2014). Moreover, ENM-dependent differential biphasic dose-response has been observed, likely due to underlying molecular mechanisms that are driving physicochemical influence on the ENM behavior (Iavicoli et al., 2018). This additional layer of complexity cannot be parsed simply by concentration; that is, some ENMs display non-monotonicity in toxic response across increasing concentrations (Bell et al., 2014). For example, responses to the silver silica composites 1%Ag-SiO₂ and ZnO are increasing and monotonic, whereas moderate toxicity for iron and aluminum (Fe₂O₃ and Al₂O₃, respectively) is only present for a single mid-range concentration (Figure S2).

ENM surface area has been linked to overall toxic response, with the most notable reasoning being the availability of a larger surface area for interactions when the mass per volume is held constant (Lee et al., 2018; Truong et al., 2019). We observe that the two ENM with the highest surface area, 1%Ag-SiO₂ and 10%Ag-SiO₂ exhibit the highest toxicity values. However, toxicity does not increase consistently with surface area, with Ag nanoparticles and ZnO exhibiting higher toxicity than Fe₂O₃ and CeO₂.

We observe this same inconsistency for circularity. Among the most toxic ENMs in our dataset, circularity measures close to 1. However, this relationship is not upheld in the less toxic materials, where circularity is more varied. The shape itself is not a distinct predictor of toxicity, with surface area and polydispersity playing an interactive role with the shape. Given the relationships between surface area, pore size, and conductance, it is likely that in some instances, ionic content is driving toxicity, while in others, the ionic content is too low or uninfluential on the toxic response.

Similarly, although particle agglomeration will affect the particle size distribution *in vivo*, PDI shows no definitive pattern when mapped to toxicity. One explanation is that initial PDI measurements will change *in vivo* due to interactions with proteins in serum that cause the development of a protein corona. The structure of the protein corona depends on size and shape, so our selected PCC may be indicative of protein corona composition and conformation (García-Álvarez et al., 2018). This highlights the importance of understanding the interactive behaviors amongst PCC which can suppress or enhance a toxic response. So, although observed toxicity across the 4 variables does not show any consistent pattern, the success of the four-variable model demonstrates their interactive behavior in driving toxicity.

3.6. Validation Set Presents Similar Pattern of Predictive Behavior

To assess the performance of the random forests method on predicting wAggE, we used a validation set containing measurements for surface area and polydispersion to form a three-variable model including concentration (ppm). When applying this model to prediction validation set toxicity measurements, we find that the MSE between models are comparable ($MSE_{Validation,3Var} = 1.103$; $MSE_{Validation;CONC} = 1.106$), whereas the R^2 value for the three variable model is small and less than that of the concentration-only model ($R^2_{Validation,3Var} = 0.003$; $R^2_{Validation, CONC} = 0.39$). Because MSE measures differences between observed and predicted values and R^2 measures the correlation of these values, the results indicate that inaccuracies with the three-variable model are caused by underprediction for 10nm and 20nm Ag nanoparticles at higher concentrations. However, the structure of the observed vs. predicted values are consistent with our training data with the concentration-only model over-predicting wAggE for ENMs under the toxicity threshold (Figure 3). Because of the similarities between the four-variable and three-variable model, it is unlikely that the exclusion of a circularity measurement is the cause of the comparability between the three-variable and concentration models. A major limitation of the validation set is that although the materials lie within the applicability domain, the variation among the PCC measurements is comparably low and the spread of the measured wAggE values is not as diverse as the training set. However, this dataset is unique to existing datasets in that it measures *in vivo* developmental toxicity. Further validation of our model would require another dataset that expands to these parameters.

4. Discussion

In our current work, we identified 1 experimental variable (concentration) and three physicochemical characteristics (surface area, circularity, dispersion) as most relevant to the

toxicity endpoint. When we consider the contributions of each variable to our prediction, we can begin to understand the mechanisms behind the toxic behavior of these ENM.

The four variable model performance was statistically equivalent to the full model. This result indicates that the four variables can sufficiently capture information lost by excluding other features. The excluded features were shown to be largely exchangeable in terms of inclusion metrics estimated from the full dataset – suggesting that underlying correlation patterns manifest in information redundancy. The decreased performance when comparing the four variable and concentration-only model can be attributed to the modification of the concentration profile by information captured by surface area, dispersion, and circularity. The correlation of surface area and the concentration (in mass per volume) is high (Truong et al., 2019) when considering the ENM particle size. In a volume of 100 μL and a set mass of the ENM, the larger diameter particles will have higher surface area and more catalytic sites for reactions to occur (Teeguarden et al., 2007). Additionally, the dispersion of the ENM (measured by PDI) can impact what particles are available to interact with the developing zebrafish. Previously, we identified that toxicity of gold nanoparticles changed depending on the dispersion using a surfactant (Ginzburg et al., 2018). The presence of the surfactant created a corona around the ENM and increased the toxicity. This has also been observed in *in vitro* systems where, a protein corona will form when host proteins and biomolecules adsorb to the surface of the ENM. This protein corona is known to drive the *in vivo* behavior of ENM and can alter pre-corona properties (Moustaoui et al., 2019; Nierenberg et al., 2018; Rahman et al., 2013).

Another PCC that may warrant additional consideration in future studies is the core composition of the ENM. Although not always applicable for ENMs, our results when including core material did not show statistically significant differences in predictions for the training set and caused statistically significant decreases in model performance with the validation set. The ionic form of certain core material is toxic (Arai et al., 2015). However, this information is known only for well-studied core material, and it is not routine to measure the ion release in the media. The ion release could vary depending on the media (Bizmark and Marios, 2015). Therefore, the inclusion of this parameter in the model could add uncertainty when there is no analytical measurement.

The underwhelming performance of our model on the validation set reiterates the need for determining ENM PCCs that contribute to toxicity. Here, we have identified the PCCs that have a quantitative relationship with the developmental toxicity endpoint using a machine learning technique commonly applied within predictive modeling schemes. However, to develop a fully capable predictive model would require not just PCCs, but structural and molecular information.

In terms of our model, the complicated interplay of ENM PCC presents a non-monotonic and non-linear relationship with toxicity. The features selected as most informative describe aspects of the dynamic interplay between ENMs and biological systems. Indeed, there was statistically insignificant performance trade-off in moving from the full to the 4-variable model. The appeal of a parsimonious model is due, in part to facilitate meta-analysis and recombination with other datasets. The characterization of PCC for nanomaterial data is an

active area of research in its own right, so models that require the fewest descriptors possible are desirable for decreasing study-to-study variability and increasing similarities in heterogeneous datasets for the development of a larger-scale modeling effort.

5. Conclusion

With this analysis, we have shown that *in vivo* ENM toxicity can be modeled by a small set of measurable PCC. Patterns within and across these imply dynamic interactive effects that affect the underlying molecular mechanisms driving toxicity. Thus, those attempting to design or utilize non-toxic ENMs should consider combinations of both measured and computationally-derived features. While our model achieved solid performance, we acknowledge the moderate size of the training set and the diversity of the selected ENMs within our model. As the amount of ENM data grows, random forests decision trees may be useful for such an exercise, as inherent groupings of ENMs can be spliced within new data to predict toxicity. In conclusion, our study reinforces the need for data sharing in the ENM domain, where results from multiple test systems and diverse feature spaces can be recombined to derive solid predictive models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ENM	Engineered Nanomaterial
hpf	Hours Post Fertilization
MSE	Mean squared error
PCC	Physicochemical Characteristic
PDI	Polydispersion Index
QSAR	Quantitative Structure-Activity Relationship
ROC	Reactive Oxygen Species
wAggE	weighted aggregate entropy
%IncMSE	Percent Increase Mean-Squared Error

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Highlights

- Random forests decision tree model identifies surface area, circularity, and polydispersion index as important variables for characterizing nanomaterial developmental toxicity.
- Concentration, surface area, circularity, and polydispersion index capture developmental toxicity equivalently to the full 14 variable set.
- Developmental toxicity is driven by dynamic interactive effects of nanomaterial characteristics.

Figure 1.

Random Forests decision tree variable inclusion statistics. Concentration was used for the majority of split rules and was the variable included in the most resampled tree models. (A) The horizontal axis measures the proportion of split rules (out of 19484) per variable. Bars are labeled with the total number of split rules. (B) Number of models in the sum-of-trees model including each variable.

Figure 2.

Over and underprediction by ENM for each model. For each model (column) and ENM (row), the predicted wAggE is measured along the vertical axis and the observed wAggE is measured on the x-axis. Reported MSE are calculated as departures in the predicted wAggE from the observed wAggE. Gold points indicate ENMs with underpredicted wAggE and blue points indicate ENMs with overpredicted wAggE. The concentrations at which wAggE was measured is indicated by opacity. The diagonal line separates under and over prediction and the dotted line is at the $\chi^2_{0.5} = 2.37$ threshold.

Figure 3.
Validation set over and underprediction by ENM for each model.

Table 1

The fourteen engineered nanomaterials tested.

Material Name	Abbreviation
Silver Silica Composite 1% w/w Silver	1%Ag-SiO ₂
Silver Silica Composite 10% w/w Silver	10%Ag-SiO ₂
20 nm Silver Nanoparticles	Ag
Ag 20 nm NPs Citrate Capped (in suspension)	Ag-Cit
Aluminum Oxide 30 nm NPs (in powder form)	Al ₂ O ₃
Au 15 nm NPs Citrate Capped (in suspension)	Au-Cit
Cadmium Sulfide Nanoparticles (Powder form)	CdS
Cerium (IV) Oxide 10 nm NPs	CeO ₂ -10nm
Cerium Oxide (IV) CeO ₂ 30nm	CeO ₂ -30nm
Iron (III) Oxide 10 nm NPs	Fe ₂ O ₃
MgO Nanoparticles (Powder form)	MgO
Silicon Oxide 15 nm NPs (Powder form)	SiO ₂
TiO ₂ Degussa P25 (Powder form)	TiO ₂ -P25
Tungsten Oxide Nanoparticles (Powder form)	WO ₃
Zinc Oxide Nanoparticles (Powder form)	ZnO

Table 2

Numeric distributions for each physicochemical characteristic. The minimum, mean (average), and maximum values for the ENM set are presented

	Minimum	Mean	Maximum
Aspect Ratio	1.10	1.21	1.44
Circularity	0.75	0.91	1.00
Conductance (mS/cm)	2.99	9.88	13.20
Median Feret Diameter (nm)	7.50	20.37	42.80
Effective Density (g/cm ³)	1.07	3.35	19.30
Hydrodynamic Diameter (nm)	36.96	316.72	852.2
Polydispersity Index	0.11	0.27	0.45
Average Pore Size (nm)	1.70	5.02	11.49
Total Pore Volume (cm ³ /g)	0.05	1.13	6.36
Roundness	0.74	0.83	0.92
Surface Area (m ² /g)	15.95	102.85	359.82
Zeta Potential (mV)	−17.70	−12.82	−9.56
	Yes	No	
Coating	2	13	

Table 3

Distribution of wAggE for each ENM across concentration

	Concentration					
	0	2.32	5	10.7	23.2	50
1%Ag-SiO2	0.21	1.74	5.91	7.42	7.77	8.42
10%Ag-SiO2	0.21	6.27	5.85	6.23	6.6	6.6
Ag	1.18	0.98	3.58	4.07	3.71	5.27
Ag-Cit	1.38	7.39	11.4	7.3	6.6	6.6
Al2O3	0.54	0.8	1.84	1.03	0.93	1.15
Au-Cit	1.03	1.86	0.82	0.41	0.97	0.62
CdS	1.24	1.19	0.73	1.65	1.77	1.75
CeO2-10nm	1	0.91	0	0.41	1.44	1.61
CeO2-30nm	0.41	0.62	0.82	0.82	0.82	1.44
Fe2O3	0.21	0.21	0.21	1.4	0.21	0.59
MgO	0.7	0.62	0.56	0.8	1.64	1.1
SiO2	0.41	0.92	1.26	0.21	0.62	0.78
TiO2-P25	0.21	1.55	0.82	1.42	1.01	0.21
WO3	0.79	0.73	0.21	1.06	0.71	0.21
ZnO	0.41	1.22	0.96	3.63	5.04	6.6

Table 4

Variable Importance. Variables are sorted by calculated variable importance. Percent increase in MSE measures how model accuracy decreases when a single variable's values are permuted.

	% Increase MSE
Circularity	22.45
Polydispersion	19.79
Surface Area (m ² /g)	16.44
Concentration (ug/mL)	16.14
Roundness	14.87
Conductance (mS/cm)	14.24
Effective Density (g/cm ³)	13.41
Median Feret Diameter (nm)	12.56
Total Pore Volume (cm ³ /g)	9.67
Zeta Potential (mV)	8.93
Hydrodynamic Diameter (nm)	8.49
Aspect Ratio	7.70
Average Pore Size (nm)	5.80
Coating	5.14

Table 5

Decision tree performance metrics for each model. Out-of-bag Mean Squared Error (MSE) and R^2 values are reported for each model

	Train		Validation	
Model	MSE	R^2	MSE	R^2
<i>Full</i>	2.37	0.62		
<i>Circularity + PDI + Concentration + Surface Area</i>	1.96	0.68		
<i>PDI + Concentration + Surface Area</i>	1.92	0.689	1.103	0.003
<i>Concentration only</i>	6.48	−0.05	1.106	0.39