



Machine Learning-Based Prediction of Metal Oxide Nanoparticle Cytotoxicity Using Physicochemical and Biological Characteristics

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Abstract

Metal oxide nanoparticles are widely employed in biomedical, pharmaceutical and nanotechnological fields, but their toxicity is highly dependent on the physicochemical characteristics, exposure conditions and biological context. Machine-learning methods offer a practical pathway towards incorporating these diverse features into predictive toxicity assessment. The data set was curated and analysed using a subset of metal oxide nanoparticles after endpoint filtering, duplicate removal, leakage control, categorical encoding, scaling, and stratified train-test partition. The following models were tested in this study using cross validation and independent test performance: Logistic Regression, Random Forest, Support Vector Machine, Gradient Boosting and XGBoost. Logistic Regression had the best test ROC-AUC value of 0.8165 and the most consistent cross-validation discrimination, while Gradient Boosting had the highest test accuracy of 0.7627, a test recall of 0.9762, and a test F1-score of 0.8542. Using SHAP analysis, oxidative potential, dissolution rate, impurity content, release of Zn^{2+} ions, zeta potential, particle size, nanoparticle identity and exposure-related characteristics were identified as key factors contributing to the prediction of cytotoxicity in the ExAI analysis. The biological relevance of the computational results was further corroborated by the strong correlation between the biological processes of membrane damage, apoptosis, necrosis and reduction in cell viability. The modelling framework excluded direct toxicity-response indicators from the primary predictor set, which minimized the leakage of targets and gave a more realistic estimate of predictive performance. The findings highlight the importance of interpretable machine learning in nanotoxicity screening, material prioritisation and safer-by-design development of nanoparticles, and the need for larger harmonised datasets and external independent validation.

Keywords: Metal Oxide Nanoparticles; Cytotoxicity; Machine Learning; Nanotoxicology; Explainable Artificial Intelligence; Physicochemical Properties

1. Introduction

The biomedical and pharmaceutical applications of metal and metal oxide nanoparticles are gaining significance due to their small size, high surface area-to-volume ratio, tunable surface properties, and functional characteristics, which include drug delivery systems, diagnostics, antimicrobial systems, imaging, and other therapeutic technologies. Concurrently, their use has grown, raising concerns about their biological compatibility and possible cytotoxicity. Nikzamir et al. (2021) pointed out that, in addition to their biomedical applications, nanoparticles could have harmful effects on cells and thus, reliable toxicity assessment is a crucial part of the development of nanomaterials. There is no single property that determines the toxicity of metal oxide nanoparticles. The size, morphology, surface area, surface charge, aggregation state, dissolution behavior, coating and chemical composition may affect the uptake and biological reactivity of cells. The physical state and physicochemical properties of metal and metal oxide nanoparticles have a significant influence on their toxicological properties, as pointed out by Manuja et al. (2021). Gakis et al. (2023) also demonstrated that toxicity must be understood in a wider framework of structure–activity relationships, with the properties of the materials and the exposure conditions and biological context. The interactions can affect oxidative stress, ion release, membrane damage, apoptosis, and loss of cell viability. Traditional nanotoxicity testing uses *in vitro* and *in vivo* experiments. While these methods have proven useful in the lab, they are often labor intensive, costly, and hard to adapt to the growing number of different formulations and exposure conditions of nanoparticles. This growing corpus of curated nanotoxicity data has thus provided opportunities for computational methods that can complement experimental testing and aid in hazard screening at an early stage. Estimation of the cytotoxicity of nanoparticles by measurable descriptors using Nano-QSAR and related modelling strategies has been gaining in popularity. Li et al. (2022) showed the increasing importance of nano-QSAR for metal and metal oxide nanoparticles, especially for the correlation of physicochemical properties with biological effects. More recently, AI and machine-learning techniques have taken this a step further and allowed for the detection of nonlinear patterns and prediction of multiple variables. Banaye Yazdipour et al. (2023) noted the growing adoption of AI tools for predicting the toxicity of nanoparticles, and Zhou et al. (2024) highlighted machine learning as a promising area in nanotoxicology but also acknowledged the ongoing challenges in the field, such as data quality, interpretability, heterogeneity, and model generalizability. One of the most important research needs is thus to create predictive models that combine the physicochemical properties, exposure conditions, and biological context without information leakage from variables that directly define the toxicity outcome. This is especially crucial if the aim is to not only achieve high predictive performance but also to determine the material properties that are most strongly linked with cytotoxicity. The goals of this work were to characterize the physicochemical, exposure related, and biological properties associated with metal oxide nanoparticle cytotoxicity, to develop and compare machine-learning models for binary cytotoxicity prediction, to assess model discrimination, classification performance, and probability calibration, and to identify the major physicochemical and experimental drivers of toxicity using explainable artificial intelligence.

2. Literature Review

Oxidative stress, ionic dissolution, disruption of membranes, mitochondrial dysfunction, apoptosis and necrosis are the various interconnected mechanisms by which metal oxide nanoparticles are cytotoxic. They have been shown to be toxic to various materials and under different experimental conditions. CuO nanoparticles, for instance, have been linked to oxidative damage, ion-mediated toxicity, as well as biomedical applications due to their antimicrobial and functional characteristics (Naz et al., 2023). Similarly, the biological effects of silica nanoparticles are dose- and

particle size-dependent and are context-dependent. Ding et al. (2023) identified mechanisms of oxidative stress, inflammation, membrane interaction, and intracellular response, while Janjua et al. (2023) identified that the safety of silica nanoparticles is closely related to their physicochemical properties and interaction with biological barriers. Another material that has been extensively studied is ZnO nanoparticles, where toxicity is dependent on the dissolution, Zn^{2+} release, particle size, surface properties and exposure conditions (Canta & Cauda, 2020). These observations show that the toxicity of a nanotechnology is not just a function of the material. Biological response is a function of the interaction of particle size, surface area, zeta potential, dissolution rate, ion release, surface chemistry, coating, exposure dose, and exposure duration. Smaller particles can have higher surface reactivity and surface charge can affect cellular interaction and uptake. Dissolution can also be responsible for an increase in toxicity due to the liberation of metal ions. Roy and Roy (2022) correlated the characteristics of metal oxide nanoparticles with toxicological results in order to elucidate the relationship between the two and to fill the gaps in nanomedicine risk assessment using mechanistic QSAR methods. In addition, Na et al. (2023) showed the usefulness of nano-QSAR modelling for predicting the toxicity of metal oxides, further emphasizing the role of physicochemical descriptors in quantitative toxicity assessment. These structure–toxicity relationships have been expanded using machine-learning strategies, where several descriptors can be analysed at the same time. Sayed et al. (2024) presented a study on the use of machine-learning algorithms to predict the toxicity of metal oxide nanoparticles, illustrating how computational models can aid in the classification of nanoparticles based on their measured properties. A machine learning approach within a meta-analytical framework was also applied by Masarkar et al. (2024), who showed the benefit of merging data from several different studies on nanoparticles to better predict cytotoxicity. Ahmadi et al. (2024) compared the toxicity prediction of nanoparticles using machine-learning methods and demonstrated that the prediction performance is highly dependent on the model used, the representation of the features, and the properties of the data set available. The results of these studies suggest that the quality of the descriptors is as crucial as the complexity of the algorithm, and that biological relevance is equally important. Nano-QSAR is also gaining increasing significance for the modelling of more complex toxicological situations, such as nanoparticle mixtures. Zhang et al. (2023) created machine-learning-based QSAR models to predict the toxicity of mixtures of nanoparticles, showing that effects of mixtures are not adequately modeled by models that incorporate interactions between multiple material and exposure properties. Despite these advances, there are still some drawbacks, such as heterogeneity in data, inconsistencies in experimental protocols, poor external validation, possible target leakage, and poor model interpretability. There is therefore a need for continued research to incorporate physicochemical properties, exposure conditions and biological context in a clear and transparent predictive framework, that not only classifies the material but also gives insight into the mechanisms. Such integration is crucial for enabling practical nanotoxicity screening and safer nanomaterial design via machine learning.

3. Materials and Methods

3.1 Dataset Description

This study employed the Machine Learning-Ready Dataset for Cytotoxicity Prediction of Metal Oxide Nanoparticles created by Savarkar et al. (2025). The data set had 23 attributes and 308 records, with one of the attributes being a non-informative index attribute (Unnamed: 0). The variables that remained after discarding this index column were those that described the identity of the nanoparticles, their physicochemical properties, the conditions of exposure, the context of the biology, the measurement of the cellular response, and the classification of the toxicity. The main physicochemical parameters were core size, surface area, zeta potential, aggregation state, dissolution

rate, ion release, impurity content, surface chemistry, coating type and oxidative potential. The experimental variables were dosage and duration of exposure, and the biological variables were cell type, ROS production, membrane damage, apoptosis, necrosis, IC50 and cell viability. Toxicity Class was the main outcome variable. The class distribution was 217 Toxic, 89 Non-Toxic and 2 Bactericidal observations. The bactericidal entries were not used for the binary classification task as they were not the same endpoint as the cytotoxicity. The last modelling problem was thus directed towards separating the Toxic from Non-Toxic nanoparticle observations. A summary of the analytical role of the dataset variables is provided in Table 1.

Table 1. Dataset Variables and Their Analytical Roles

Variable group	Example variables	Data type	Analytical role
Nanoparticle identity	Nanoparticle type	Categorical	Material descriptor
Physicochemical properties	Core size, zeta potential, surface area, dissolution rate, ion release	Numerical	Primary predictors
Surface characteristics	Shape, aggregation state, surface chemistry, coating type, oxidative potential	Categorical	Primary predictors
Exposure conditions	Exposure dosage, exposure time	Numerical	Primary predictors
Biological context	Cell type	Categorical	Predictor
Biological-response indicators	ROS, membrane damage, apoptosis, necrosis	Numerical	Secondary biological indicators
Cytotoxicity indicators	IC50, cell viability	Numerical	Secondary response indicators
Outcome	Toxicity Class	Categorical	Classification target

Predictive descriptors and variables that reflect cellular damage were distinguished. Biological-response variables like production of ROS, membrane damage, apoptosis, necrosis, IC50 and cell viability were retained for descriptive interpretation and not included in the primary predictor set to minimize the risk of target leakage.

3.2 Data Preprocessing

The data was preprocessed in a constant order prior to model training. First, the index column was removed and then the data was checked for missing data, duplicate records, inconsistent categorical labels, and implausible numerical entries. Distributional skewness and extreme values were checked for numerical variables and categorical fields were standardized to ensure consistent representation across observations. Numerical values missing from the dataset were filled in with the median, and the parameters of the median were calculated from the training data only. One hot encoding was performed for categorical variables. Continuous features were standardized for algorithms that are sensitive to the scale of the features, such as Logistic Regression and Support Vector Machine. The train-test split was done prior to imputation, encoding, or scaling procedures to avoid information leakage. The data was split into two groups: 80% train and 20% test, with the same proportion of Toxic and Non-Toxic cases in each group. Five-fold stratified cross-validation was then used to develop the model and select the hyperparameters in the training partition. The independent test set was not disturbed until the final model was decided. The entire pre-processing protocol is displayed in Table 2.

Table 2. Data Preprocessing and Modelling Protocol

Step	Procedure	Purpose
Endpoint filtering	Remove bactericidal observations	Maintain a consistent cytotoxicity endpoint
Index removal	Remove Unnamed: 0	Eliminate non-informative data
Data audit	Check missing values, duplicates and variable types	Data-quality control
Category harmonization	Standardize categorical labels	Ensure consistent encoding
Leakage control	Exclude direct cellular-response variables from primary prediction	Avoid artificially inflated performance
Imputation	Training-derived median imputation	Handle missing numerical values
Encoding	One-hot encoding	Convert categorical predictors
Scaling	Standardize continuous features where required	Support scale-sensitive models
Data split	Stratified 80:20 train-test split	Preserve class proportions
Validation	Five-fold stratified cross-validation	Model comparison and tuning
Final testing	Evaluate selected model on untouched test set	Estimate generalization

3.3 Exploratory Data Analysis

Exploratory data analysis was conducted to describe the data prior to machine-learning modelling. Class frequencies were first analyzed to determine if there was an imbalance between Toxic and Non-Toxic observations. Measures of central tendency and dispersion such as mean, standard deviation, median, minimum and maximum values were used to summarize continuous variables. The distributions of the features were examined for skewness and outliers. Correlation analysis was used to investigate relationships between the numerical variables, and grouped comparisons to examine the differences between the various types, shapes, coatings and cell types of nanoparticles. Special focus was paid to core size, zeta potential, surface area, dissolution rate, exposure dosage, exposure time, ROS production, IC50 and cell viability. Exploratory analysis was employed to discover potential informative relationships, redundant variables and nonlinear patterns that may affect further model development.

3.4 Machine Learning Models

Five supervised classification algorithms were selected to provide a balanced comparison across linear, kernel-based, bagging, and boosting approaches:

- Logistic Regression
- Random Forest
- Support Vector Machine
- Gradient Boosting
- XGBoost

The baseline model was logistic regression, which is an interpretable model. Random Forest and Gradient Boosting were added to account for nonlinear relationships and feature interactions. The kernel-based class separation was evaluated using Support Vector Machine and XGBoost was added as a regularized boosting method that can be used to model complex interactions between predictors. The same training data and pre-processing pipeline were used for all models. Five-fold stratified cross validation was used to select hyperparameters, but no tuning was done on an independent test set.

3.5 Model Evaluation

Model performance was evaluated using multiple complementary metrics because the dataset showed an unequal distribution of Toxic and Non-Toxic observations. The primary metrics were accuracy, precision, recall, F1-score, specificity, ROC-AUC, and confusion-matrix statistics.

Accuracy was calculated as:

$$\text{Accuracy} = \frac{TP+TN}{TP+TN+FP+FN} \quad (1)$$

Precision was calculated as:

$$\text{Precision} = \frac{TP}{TP+FP} \quad (2)$$

Recall was calculated as:

$$\text{Recall} = \frac{TP}{TP+FN} \quad (3)$$

The F1-score was computed as:

$$F1 = 2 \times \frac{\text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}} \quad (4)$$

Specificity was calculated as:

$$\text{Specificity} = \frac{TN}{TN+FP} \quad (5)$$

where TP, TN, FP, and FN represent true positives, true negatives, false positives, and false negatives, respectively. ROC-AUC was used to assess the overall discriminatory ability of each model across different classification thresholds. Cross-validation mean and standard deviation were also reported to assess model stability. The best-performing model was selected using the combined evidence from F1-score, ROC-AUC, and cross-validation consistency rather than accuracy alone.

3.6 Explainable AI Analysis

To find the variables with the greatest impact on predicting cytotoxicity, explainable AI was added. Selected model was interpreted using SHapley Additive exPlanations (SHAP). The importance of predictors was ranked by means of Global SHAP analysis and the direction of SHAP values was used to identify which direction (higher or lower) would increase or decrease the predicted probability of toxicity for the specific feature. The physicochemical properties including particle size, zeta potential, surface area, dissolution rate, ion release, coating property, exposure dosage and exposure duration were focused. The effects of physicochemical variables, exposure variables, and biological-context variables were also compared at the group level. Furthermore, individual SHAP explanations were analyzed for representative Toxic and Non-Toxic predictions to demonstrate the interplay of combinations of nanoparticle characteristics. Excluded from the primary model were biological-response variables which were used for interpretation instead of prediction, and model-derived patterns of toxicity were compared to observed cellular-response indicators without target leakage.

4. Results

4.1 Dataset Characteristics

After removing the index field, discarding the two bactericidal observations and deleting 15 exact duplicate records, the final binary cytotoxicity data set consisted of 291 observations, 207 of which were Toxic (71.1%) and 84 were non-toxic (28.9%). The data comprised physicochemical descriptors, exposure variables, biological-context information, cellular-response indicators, and the final toxicity class. Table 3 summarizes the descriptive statistics for the main numerical variables.

Table 3. Descriptive Statistics of Major Physicochemical, Exposure, and Biological Variables

Variable	N	Mean	SD	Median	Minimum	Maximum
Core Size (nm)	291	83.10	379.92	30.00	6.00	5000.00
Zeta Potential (mV)	289	-24.00	12.35	-25.00	-72.00	63.20
Surface Area	278	169.75	162.74	110.00	-10.00	1310.00

(m ² /g)						
Dissolution Rate (mg/L)	291	3.76	3.97	1.10	0.23	15.00
Zn ²⁺ Ion Release (mg/L)	291	1.78	2.74	0.00	0.00	10.00
Impurity Content (%)	291	0.037	0.036	0.050	0.002	0.200
Exposure Dosage (µg/mL)	291	5273.03	62060.74	50.00	0.91	750000.00
Exposure Time (h)	291	56.76	204.73	24.00	1.00	2016.00
ROS Production	291	91.30	77.10	60.00	0.00	300.00
Membrane Damage (%)	291	36.34	21.60	35.00	0.00	85.00
Apoptosis (%)	291	23.26	17.03	20.00	0.00	70.00
Necrosis (%)	291	12.71	10.93	12.00	0.00	100.00
Cell Viability (%)	291	60.27	24.29	55.00	0.00	100.00

There was considerable variation in particle size, exposure dosage, exposure time and some biological-response variables. The core size was in the range of 6 to 5000 nm and the exposure dosage was 0.91 to 750000 µg/mL. For some of the variables, there was a significant right skewness between the mean and median. The distribution of the main continuous variables is shown in Figure 1.

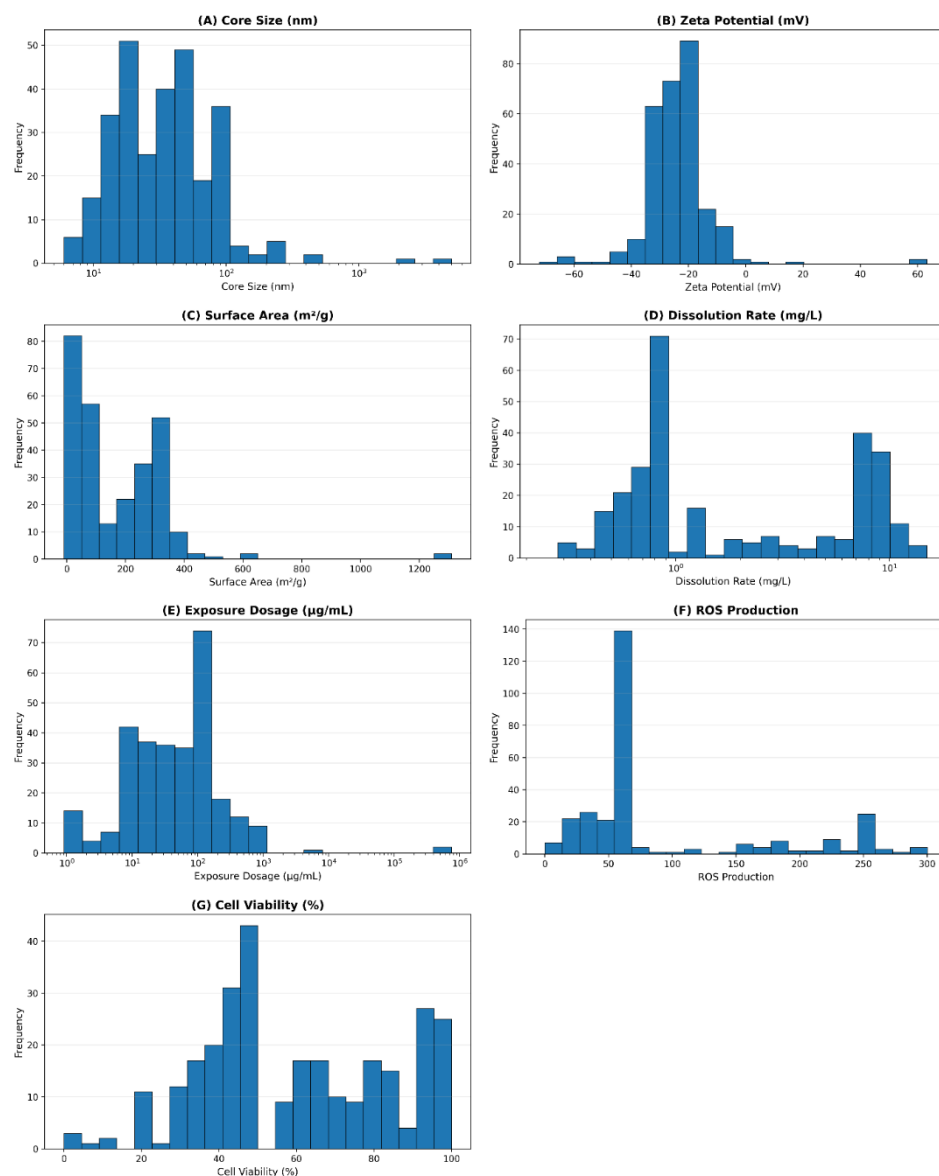


Figure 1. Distribution of Major Physicochemical, Exposure, and Biological Variables in the Cytotoxicity Dataset.

4.2 Toxicity Patterns Across Nanoparticle Characteristics

The number of Toxic and Non-Toxic observations differed between the types of nanoparticles. The most common materials in the cleaned data set were ZnO, CuO, SiO₂ and AgO. They are toxic and their distribution is shown in Table 4.

Table 4. Toxicity Distribution Across Major Nanoparticle Types

Nanoparticle	Non-Toxic, n	Toxic, n	Total, n	Toxic (%)
ZnO	18	73	91	80.2
CuO	25	43	68	63.2
SiO ₂	16	46	62	74.2
AgO	17	31	48	64.6
Ag ₂ O	2	4	6	66.7
Other nanoparticle types	6	10	16	62.5
Overall	84	207	291	71.1

As shown in Table 4, ZnO exhibited the highest toxicity proportion among the major nanoparticle groups at 80.2%, followed by SiO₂ at 74.2%. AgO and CuO showed toxicity proportions of 64.6% and 63.2%, respectively. Spearman correlation analysis identified several strong relationships among biological-response variables. Necrosis was strongly inversely correlated with cell viability ($\rho=-0.936$), while membrane damage was positively correlated with necrosis ($\rho=0.934$) and apoptosis ($\rho=0.919$). Membrane damage also showed a strong inverse relationship with cell viability ($\rho=-0.915$). Among physicochemical descriptors, dissolution rate was strongly correlated with Zn²⁺ ion release ($\rho=0.811$). These relationships are presented in Figure 2.

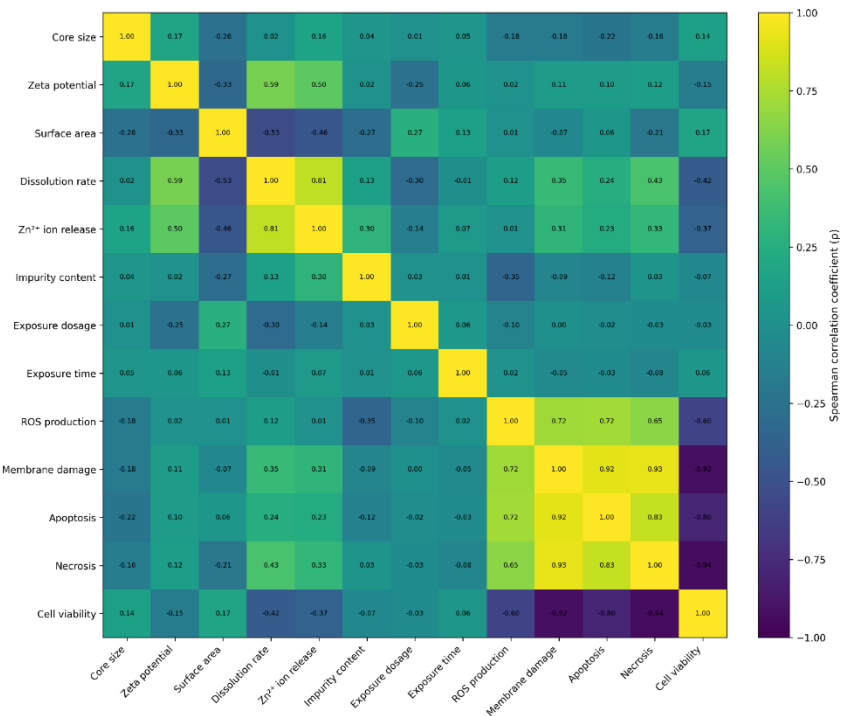


Figure 2. Spearman Correlation Heatmap of Physicochemical, Exposure, and Biological Response Variables.

4.3 Comparative Machine Learning Performance

To determine the best performing classifiers, five machine-learning classifiers were tested using five-fold stratified cross validation on the training data set, and then the results were compared to the independent test set. The comparative performance results are shown in Table 5.

Table 5. Comparative Performance of the Machine Learning Models

Model	Cross-Validation Accuracy	Cross-Validation F1-Score	Cross-Validation ROC-AUC	ROC-AUC SD	Test Accuracy	Test Precision	Test Recall	Test F1-Score	Test ROC-AUC
Logistic Regression	0.8144	0.8635	0.8860	0.0208	0.7288	0.8250	0.7857	0.8049	0.8165

Random Forest	0.7500	0.8332	0.8471	0.0449	0.6949	0.7500	0.8571	0.8000	0.7080
Support Vector Machine	0.7410	0.7865	0.8503	0.0297	0.7119	0.9032	0.6667	0.7671	0.6807
Gradient Boosting	0.8276	0.8869	0.8857	0.0482	0.7627	0.7593	0.9762	0.8542	0.7787
XGBoost	0.7844	0.8533	0.8587	0.0511	0.6610	0.7500	0.7857	0.7674	0.7269

Gradient Boosting performed best in terms of cross-validation accuracy (0.8276) and cross-validation F1-score (0.8869), whilst LR performed best in terms of cross-validation ROC-AUC (0.8860) and the lowest ROC-AUC variability (SD = 0.0208). Gradient Boosting had the best accuracy (0.7627) and F1-score (0.8542) on the test set while Logistic Regression had the highest test ROC-AUC (0.8165). The test precision of Support Vector Machine (0.9032) was high while the test recall (0.6667) was low. All evaluated models have discrimination profiles presented in Figure 3.

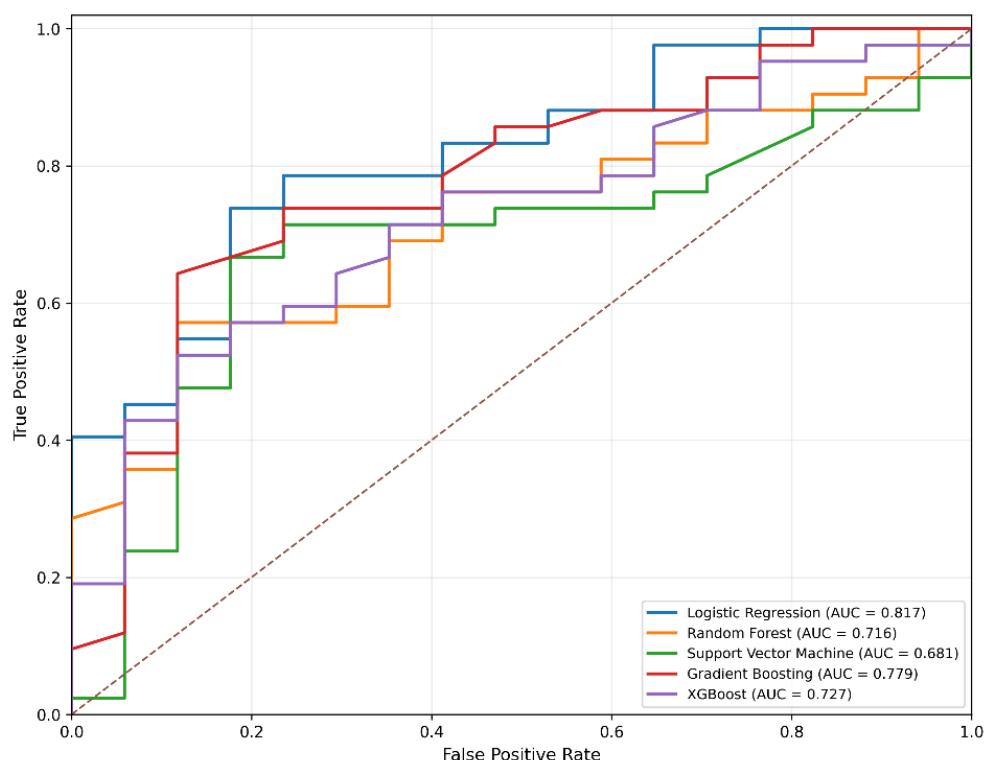


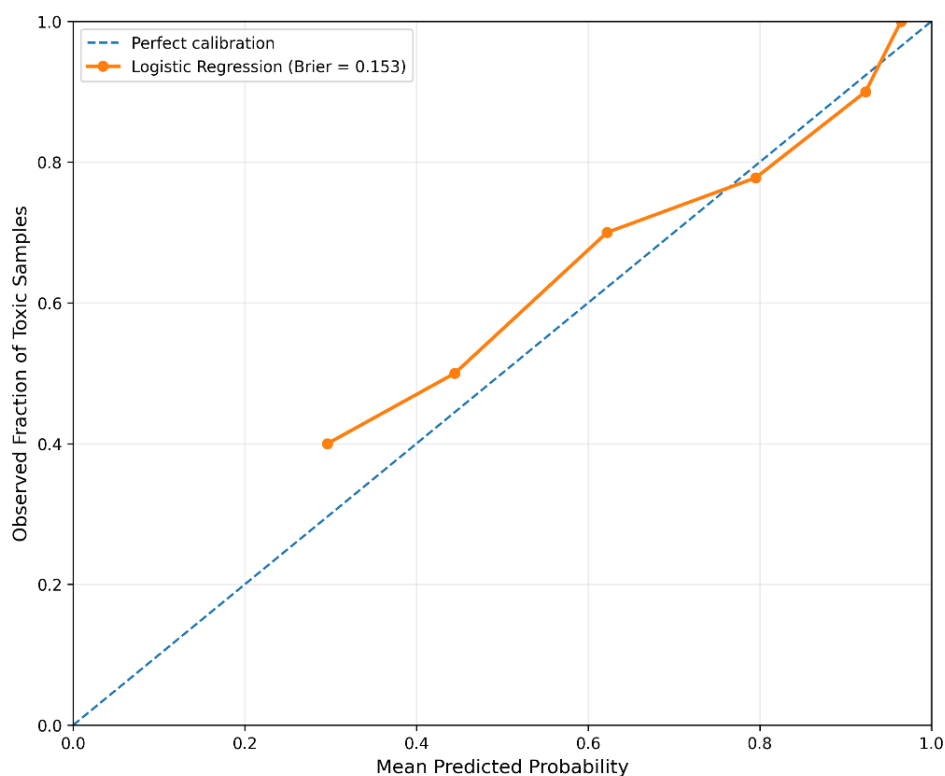
Figure 3. Receiver Operating Characteristic Curves for the Evaluated Machine Learning Models.

Logistic Regression was chosen as the main model for probability-based modelling given its maximum test ROC-AUC and the most stable cross validation ROC-AUC. The performance of detailed classification is summarized in Table 6.

Table 6. Independent Test-Set Performance of the Selected Logistic Regression Model

Measure	Result
Test samples	59
True negatives	10
False positives	7
False negatives	9
True positives	33
Accuracy	0.7288
Precision	0.8250
Recall/Sensitivity	0.7857
Specificity	0.5882
F1-score	0.8049
ROC-AUC	0.8165
Brier score	0.1527

The chosen model was able to correctly classify 33 of 42 Toxic samples and 10 of 17 non-toxic samples. Sensitivity was 78.57% and specificity was 58.82%, which implies that Toxic cases were better recognised than non-toxic cases. The accuracy was 82.50%, and the F1-score of the model was 0.8049. The selected model is calibrated to the probability in Figure 4.

**Figure 4. Calibration Curve of the Selected Logistic Regression Model for Cytotoxicity Prediction**

4.5 Explainable AI and Major Cytotoxicity Predictors

Variables with the highest contributions to cytotoxicity predictions were identified using SHAP analysis. The strongest effects were related to the oxidative potential, dissolution rate, impurities, release of Zn^{2+} ions, identity of nanoparticles, zeta potential, and core size. The dissolution rate and impurity content were the next most important contributors to model output, with the highest oxidative potential being the most important overall. The Zn^{2+} ion release also made a significant contribution, suggesting that dissolution related behaviour had a significant influence on the predicted toxicity profile. The physicochemical discrimination of identity of nanoparticles (ZnO and CuO) and zeta potential and particle size also affected predictions. The magnitude of the principal feature effects and the direction of the feature effects are shown in Figure 5.

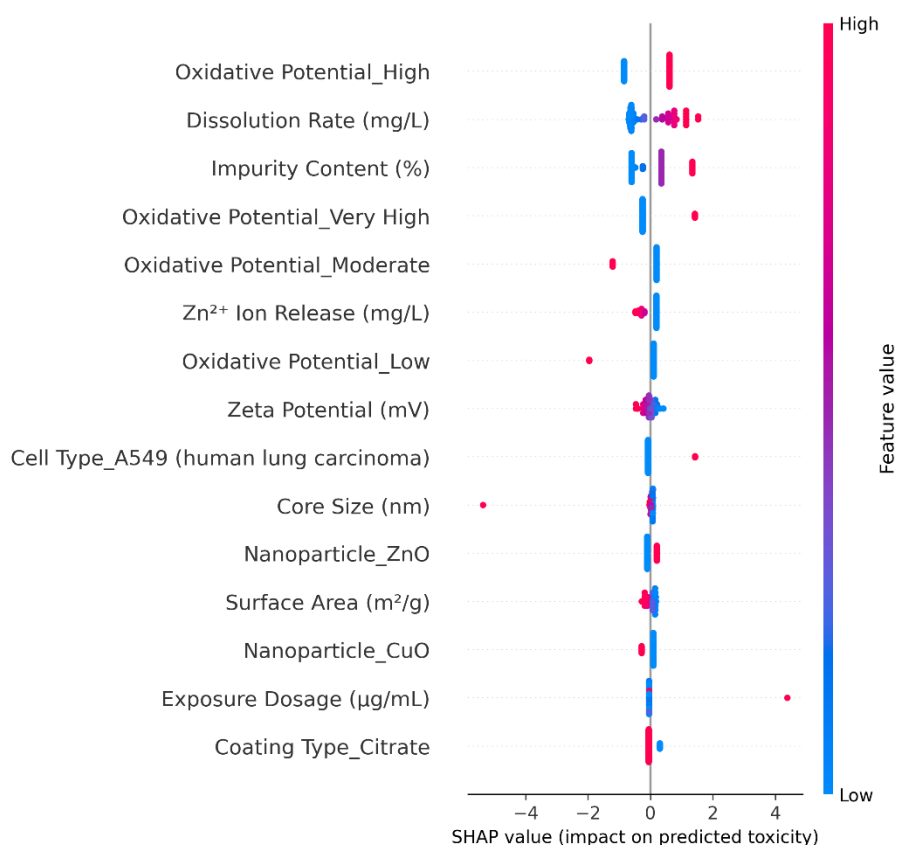


Figure 5. SHAP Summary Plot of the Major Predictors of Metal Oxide Nanoparticle Cytotoxicity

The overall modelling results suggest that the cytotoxicity could be predicted with moderately good discriminatory performance by the leakage-controlled physicochemical and exposure-related variables, and the biological-context variables. In addition, the explainability analysis revealed that the toxicity prediction was determined by the material reactivity, dissolution properties, identity of the nanoparticles, electrostatic properties, and exposure conditions, but not by any one of these descriptors alone.

5. Discussion

The modelling results suggest that metal oxide nanoparticle cytotoxicity can be successfully predicted with the combination of physicochemical, exposure-related and biological-context variables with useful discriminatory performance. Logistic Regression had the highest test ROC-AUC and Gradient Boosting had the highest test

accuracy and F1-score. This difference indicates that there were both linear and nonlinear relationships in the data. The moderate-sized and heterogeneous nanotoxicity dataset is also important, as the Logistic Regression model demonstrated relatively stable cross validation performance. The results are similar to those of previous studies by Ji et al. (2022) who showed that structured descriptors could be used to predict nanomaterial cytotoxicity using machine-learning models, with the model performance being affected by the composition of the datasets and the quality of the descriptors. The SHAP analysis showed that oxidative potential, dissolution rate, impurities, release of Zn^{2+} ions, zeta potential, particle size, and identity of the nanoparticles are the most important factors in the model predictions. These results are biologically relevant because reactivity, dissolution and ion release may affect the exposure of cells and the oxidative load, and size and surface charge may affect the interaction with biological systems. The contribution of interpretable features is important, as Yu et al. (2021) pointed out that interpretable machine-learning approaches that do not only focus on prediction accuracy are important to understand the drivers of nanoparticle cytotoxicity. The results also validate the overall structure–activity concept in the context of nano-QSAR modelling. Neither one nor several descriptors were associated with cytotoxicity, but rather combinations of physicochemical and experimental properties. This multivariable pattern is consistent with a computer-aided nano-QSAR approach proposed by Cao et al. (2020), where multiple material descriptors were used to predict toxicity. A major advantage of the present modelling approach was that direct biological-response parameters (cell viability, ROS production, apoptosis, necrosis and IC_{50}) were not included in the primary predictor set. This minimized the likelihood of leakage of the target and prevented the model from being driven by actual measurement of the toxicity endpoint itself. The biological-response variables were still useful for interpretation, however. The physicochemical properties are linked to the cell viability loss, apoptosis, necrosis and membrane damage, suggesting that the latter are related to each other and to the former. This is in line with the importance of using computational prediction in conjunction with biological measurements, as shown by Peng et al. (2020) who used machine learning and biological measurements to enhance the assessment of nanotoxicity. The results can be used in the screening of nanoparticles and in their safer-by-design development. A predictive model could be used to prioritize materials for additional experimental testing and determine combinations of properties that lead to increased risk of cytotoxicity. But there are a few drawbacks. The dataset was moderate size, the Toxic class was more prevalent than the Non-Toxic class, and the experimental conditions were different depending on the type of nanoparticle and the biological system. These factors might restrict generalizability. The model needs to be validated by an independent nanotoxicity database before it can be used for wider predictive applications.

6. Conclusion

Physicochemical, exposure-related and biological-context characteristics showed strong potential to predict the cytotoxicity of metal oxide nanoparticles with machine learning, without direct toxicity-response variables being mixed in. Logistic Regression had the highest test ROC-AUC and the most stable cross-validation discrimination, while Gradient Boosting had the highest test accuracy, recall, and F1-score, suggesting that both linear and nonlinear relationships played a role in the toxicity prediction model. The explainable AI analysis also revealed that the oxidative potential, dissolution rate, impurity content, release of Zn^{2+} ions, zeta potential, particle size, identity of the nanoparticles and exposure-related factors were the most influential factors in predicting toxicity. The results endorse the idea that the cytotoxicity of nanoparticles is not only due to the reactivity of the material, but also due to its dissolution behavior, electrostatic properties, composition, and the context in which it is tested. The close association between damage to the membrane, apoptosis, necrosis

and decreased cell viability further supports the biological validity of the computational results. The use of target leakage reduction by excluding direct biological-response indicators from the primary predictive model was a key methodological advantage of the study, as it led to a more realistic estimation of the predictive performance. The established framework can thus be used to aid in screening for nanotoxicity, selection of candidate materials for additional testing, and the development of safer-by-design nanoparticles. However, the relatively small size of the datasets, the class imbalance, and the variety of experimental conditions restrict the immediate generalisation. Future research should focus on external validation, larger harmonized data sets, expanding coverage of nanoparticles and continued work with explainable models to further enhance the trustworthiness and translatability of machine learning-based nanotoxicity prediction.

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