Hierarchy of Nanoparticles Toxicity Factors Significance as Extracted from NanoCommons Knowledge Base: Influence of Compound, Cell line and Particle Size on Cell Viability

Authors:

1. Michal Macko[1]
2. František Božek[1]
3. Diana Kmetkova[2]
4. Jan Antoš[1]
5. Ivo Kuřitka[1]

Affiliations:
1) Tomas Bata University in Zlin, Centre of polymer systems, třída Tomáše Bati 5678,
760 01 Zlín, 760 01 Zlín, Czech Republic
2) Institute of Economic Studies, Charles University, Opletalova 26, Prague, Czech Republic

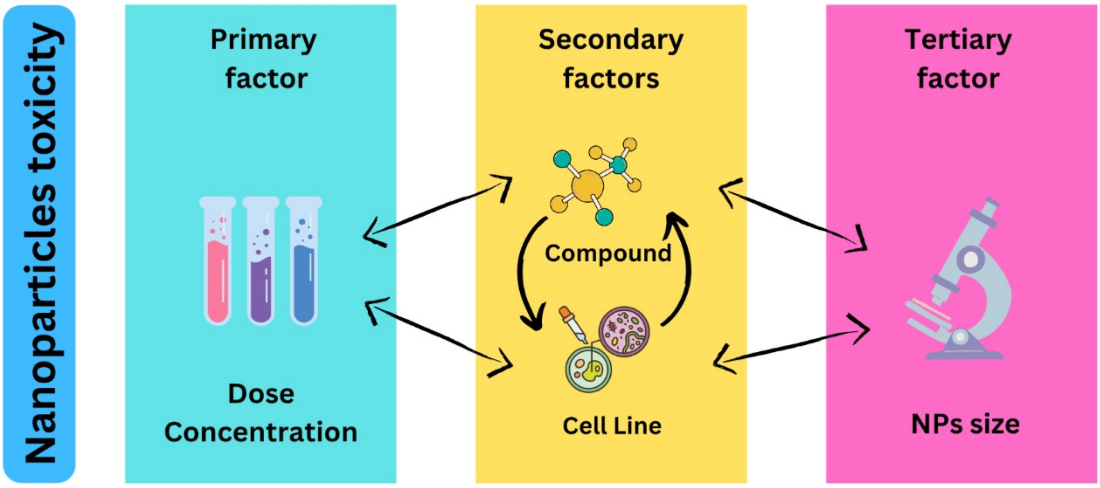
Corresponding authors: Ivo Kuřitka, Michal Macko
Address: Tomas Bata University in Zlin, Centre of polymer systems, třída Tomáše Bati 5678, 760 01 Zlín, 760 01 Zlín, Czech Republic
E-mail: kuritka@utb.cz; mmacko@utb.cz

Abstract

The objective of the paper was to conduct a thorough statistical analysis of nanoparticles of publicly available data found in the NanoCommons database (“BioXMTM Knowledge Portal." NanoCommons,” 2024), specifically, how various parameters influence cell viability value (CVV). The analysis was performed on nine hundred measurements, where required parameters (compound, dose, exposure time, cell line, nanoparticle size) were available. The data represented a collection out of seven datasets, which contained five cell lines and six compounds. Other parameters included a constant exposure time of 24h, particle diameters from 8 to 150 nm, and exposure doses ranging from 0.3 to 250 [μg/ml]. The effects of variables were explored via two main models - first model was a Multiple Ordinary Least Squares (OLS) Model without interactions (Model 1), aimed at exploring the significance of certain variables and their effect on cell viability without considering any interactions. The second was a Multiple OLS Model with interactions (Model 2), which aimed to explore the significance of certain variables on cell viability while also

considering interactions between some of the regressors. The findings emphasize the importance of analyzing compound- and cell-specific interactions, as well as the interplay between particle size and nanoparticle reactivity.

Graphical abstract



Keywords

Nanoparticles, toxicity, statistical analysis, particle size

Introduction

Nanoparticles (NPs) are defined as particles with at least one dimension ranging between 1 – 100 nm (Bezza et al., 2020). Their properties emerging due to their small size and high surface-to-volume ratio are explored in many industrial fields, such as medicine (Islam et al., 2022), electronics (Chakraborty et al., 2022), agriculture (ESKİN, 2022; Pansambal et al., 2023) or environmental science (Arun et al., 2023; Samuel et al., 2022). Besides their positive properties, they also have adverse toxicological effects on biological systems (Gupta and Xie, 2018). Therefore, the mechanisms of nanoparticle-induced toxicity are intensively researched (Gudkov et al., 2021; Naz et al., 2020; Nie et al., 2023; Thakur et al., 2024). The toxicity mechanisms depend on numerous factors. Among them, the most acknowledged are elemental composition, size, shape, or surface charge (Abbasi et al., 2023). A large amount of knowledge has been gathered but remains dispersed among individual studies. To obtain the bird's-eye view on the multiple sources of various data, an H2020 Infrastructures project, NanoCommons was executed creating a community framework and infrastructure for reproducible science, and in particular for in silico workflows for nanomaterials safety assessment and beyond (“NanoCommons: The European Nanotechnology Knowledge Infrastructure,” 2025). The NanoCommons database is a knowledge base collecting and sharing of self-consistent and reliable data on nanoparticle characterization and toxicity.

Among many others, the toxicological behaviour and toxicity mechanisms of six common compounds available as NPs are considered, aiming at the utilisation of the data from the NanoCommons database in this study: (i) oxide materials, namely titanium dioxide (TiO_2), zinc oxide (ZnO), cerium dioxide (CeO_2), silicon dioxide (SiO_2), and (ii) metals, namely silver (Ag), copper (Cu).

Titanium dioxide (TiO_2) is one of the most widely produced semiconducting nanomaterials. TiO_2 NPs possess UV-blocking and photocatalytic properties, high stability, and biocompatibility. Although TiO_2 NPs are generally considered biologically inert under dark conditions, upon photoactivation by UV light, they generate reactive oxygen species (ROS), can cause DNA damage, or lead to pulmonary toxicity after inhalation (Mohamed, 2015; Ziental et al., 2020). Another factor influencing the toxicity of TiO_2 NPs is the crystal structure - anatase vs. rutile. The observations indicate that the anatase form is more toxic due to higher photocatalytic activity leading to increased generation of ROS (Baranowska-Wójcik et al., 2020; Nosaka and Nosaka, 2017; Thakur et al., 2024).

Zinc oxide nanoparticles (ZnO NPs) are also mass-scale produced and have already been introduced in many applications utilizing their antimicrobial and UV protective functions and relatively high biocompatibility. Interestingly, enhancement of drug delivery was also reported. (Gudkov et al., 2021; Islam et al., 2022; Silva et al., 2019). On the other hand, ZnO NPs can cause oxidative stress, lipid peroxidation, DNA damage, and ultimately, cell death, which are ascribed to the formation of reactive oxygen species (ROS) or due to the release of zinc ions (Zn^{2+}) (Canta and Cauda, 2020; Chang et al., 2012; Chen et al., 2019; Fukui et al., 2012; Song et al., 2010).

Another industrial large-scale produced semiconductor nanomaterial is cerium dioxide (CeO_2). CeO_2 NPs can act both as antioxidants and prooxidants, due to their ability to switch between cerium (III) and cerium (IV) oxidation states, often used as catalyst. The nature of CeO_2 NPs allows to scavenge ROS and protect cells from oxidative damage continuously (Singh et al., 2020), and for that reason, among many others, their applications can be found in sunscreens or cosmetics. (Pansambal et al., 2023). Nevertheless, at higher concentrations, CeO_2 NPs can induce oxidative stress, cause DNA damage, or trigger immune responses and inflammation (Forest et al., 2017; Gao et al., 2014).

Silica, i.e. silicon dioxide (SiO_2) nanoparticles, is the second most widely produced oxide nanomaterial after alumina. SiO_2 NPs are widely used in food, cosmetics, and pharmaceuticals because of their biocompatible and chemically stable properties (Slowing et al., 2008). While generally recognized as considerably less toxic than other nanoparticles, SiO_2 NPs can induce toxicity associated with surface reactivity, which can lead to the generation of ROS, inflammatory responses, membrane damage, and DNA fragmentation (El-Sayed et al., 2021; ESKİN, 2022; Liman et al., 2020; Rafieepour et al., 2021; Yang et al., 2019).

Due to their exceptional antimicrobial and biocompatible properties, metal silver nanoparticles (Ag NPs) are used in many fields such as agriculture, electronics, textile, hygiene or medicine and medical devices. When it comes to the mechanism of toxicity, primarily, it is the release of silver ions (Ag^+) that induces oxidative stress. Direct nanoparticle interaction with the cell membrane was also described. Next, even in the absence of ROS, Ag NPs can cause damage to the mitochondrial function, leading to cell death (Bruna et al., 2021; Luceri et al., 2023; Nie et al., 2023; Xu et al., 2020).

Another widespread material is copper (Cu) and copper-based NPs, often in the form of metal oxide (CuO). Among others, metal Cu NPs are used as materials for superconductors in electronics or as antimicrobial agents for coatings and food preservatives (Chakraborty et al., 2022; Naika et al., 2015; Yip and Sauls, 1992). However, when they interact with biological systems, copper ions (Cu^{2+}) may be released, which may induce oxidative stress, or cause mitochondrial malfunction and DNA damage (Ahamed et al., 2010; Akintelu et al., 2020; Fahmy and Cormier, 2009; Naz et al., 2020).

The logical counterpart in the evaluation of a toxic agent is its target. The target is determined by the method used – in vitro (outside of a living organism) or in vivo (within a living organism) (Savage et al., 2019). The scope of this paper focuses on in vitro methods, specifically measurements conducted on cell lines. Each cell line used for testing represents specific tissue characteristics, metabolic capacities, and stress response mechanisms, resulting in their different sensitivity to various compounds. Therefore, selecting appropriate cell lines is critical for understanding toxicity mechanisms, predicting in vivo responses, and evaluating potential risks (Kumar et al., 2017). Among the cell lines examined in the studies collected in the NanoCommons database, five cell lines are relevant in this study, namely HepG2, HepaRG, A549, RAW264.7, and HCT116. This selection covers four important areas of human organism toxicity, i.e., toxicity to the liver, gastrointestinal tract, pulmonary system, and inflammation responses.

The widely used model for hepatic metabolism and cytotoxicity studies are **HepG2** cells which are derived from human liver carcinoma (Ahamed et al., 2020; Elje et al., 2020; Yuan et al., 2011). In particular, HepG2 cells are relevant for studying effects of nanoparticles that release metal ions when dissolving, such as ZnO, TiO_2, Ag or Cu, due to their role in metabolizing reactive compounds and detoxifying oxidative stress (Brandão et al., 2020; Brkić Ahmed et al., 2017; Liu et al., 2021; Reihani et al., 2024). Compared to HepG2, **HepaRG** (Human Hepatocellular cells) are considered a superior model for studying hepatotoxicity (Guo et al., 2020). It is due to their ability to replicate the metabolic and inflammatory responses of liver cells in the human body towards various compounds, including NPs. Furthermore, their sensitivity to oxidative stress and metal ions makes them an ideal model used for distinguishing between direct cytotoxic effects and metabolism-dependent toxicity (Helvenstein et al., 2015; Kuhn et al., 2024; Voss et al., 2020).

The response of gastrointestinal tract is modelled by **HCT116** cell line which is derived from human colorectal carcinoma. HCT116 cells are often used to study the gastrointestinal toxicity of nanoparticles, particularly those administered orally or exposed via dietary sources. These cells are highly proliferative and exhibit strong DNA repair mechanisms, which make them a robust model for studying genotoxicity and oxidative stress (Ahmed et al., 2015; Gurunathan et al., 2018; Jia et al., 2020; Vecchiotti et al., 2021).

A commonly used model for respiratory toxicity are **A549** cells which are derived from human alveolar basal epithelial cells (Ahamed et al., 2019; Ahmad et al., 2017). A unique feature of A549 cells is their suitability for testing the particle size-dependent uptake and cytotoxicity, which is due to the larger nanoparticles remaining in the extracellular space, while smaller particles are translocated deeper and absorbed by cells (Chairuangkitti et al., 2013; Kim et al., 2006; Rafieepour et al., 2022).

The model frequently used to investigate how the immune system reacts to nanoparticles are **RAW264.7** cells, derived from murine macrophages. Besides their origin, it is due to their ability to produce high level of ROS when exposed to foreign substances (Bo et al., 2023). For that reason, they are recognized as a valuable model for researching inflammation and oxidative stress brought on by nanoparticles (Dussert et al., 2020; Giovanni et al., 2015; Hsieh et al., 2015).

Data and Methods

This work aimed to subject the publicly accessible dataset from the NanoCommons database (available under Creative Commons Attribution 4.0 International (CC BY 4.0)) to statistical analysis to verify how different parameters, specifically nanoparticle diameter, nanoparticle composition, and testing method influenced toxicity scores. Initially, the whole database was examined, counting in a total of 1,631 different measurements over 10 different research studies, 12 different examined compounds, and 6 different cell lines. After a more detailed examination, it became clear that measurements were observing different parameters. For instance, there were only 19 results for mortality or malformation and only 46 results for cell death, or cell death using the s.e.m method. For that reason, it was necessary to subject the whole dataset to a more detailed review to identify which parameter had the biggest representation and was able to interpret the toxicity of the studied compounds. The list of all available parameters in the NanoCommons database is displayed in Table 1.

Table 1: List of parameters available in NanoCommons database

Parameter	Values present for given parameter
Toxicity measurement	1631
_ToxExp.Sample	1631
_ToxExp.Dataset	1631
_ToxExp.Measurement Name	1631
_ToxExp.Time	1631
_ToxExp.Dosis [µg/ml]	1631
_ToxExp.Cell	1599
_ToxExp.Particle	1575
_data.Viable Cell Count.Value	1541
_data.Cell membrane damage.Value	1273
_data.Mitochondrial membrane potential.Value	1273
_data.Nuclear Intensity.Value	1273
_data.Nuclear Size.Value	1273
_data.Lysotracker.Value	273
_data.Cytoplasmic caspase 3.Value	261
_data.Fraction of caspase negative cells.Value	261
_data.Lipid droplet size.Value	261
_data.Nuclear caspase.Value	261
_data.Total caspase.Value	261
_data.Cell Death.Value	131
_data.Cell Death Hoechst PI [%].Value	131
_data.Cell Death [% of pos control].Value	46
_data.Cell Death s.e.m.Value	46
_data.IL-8 release [pg/ml].Value	46
_data.IL-8 release s.e.m..Value	46
_data.Viability [% of negative control].Value	46
_data.Viability s.e.m..Value	46
_data.Hatching inhibition [%].Value	33
_data.Malformation [%].Value	19
_data.Mortality [%].Value	19

_data.Nuclear caspase.,	1
_data.Nuclear Intensity.,	1
_data.Nuclear Size.,	1

It was identified that the parameter with the biggest representation was data.Viable Cell Count.Value, which was present in 1,540 measurements out of 1,631, representing almost 95% coverage of the whole dataset. This parameter was selected as the main observed value, as it was also a commonly used indication of the toxicity of nanoparticles. The next step was to verify other parameters, namely: compound, exposure doses, exposed cell line, diameter of NPs, and exposure time, and check their presence in the dataset.

After closer examination, it was found that 542 measurements had no information about nanoparticle diameter, so these measurements were removed from the dataset. The next refinement was focused on compounds, specifically on different doses, sufficient count of measurements, or different particle diameters. It was found that 48 measurements represented positive control, and 50 measurements of Au NPs had only one diameter – 12 nm on one cell line – HepaRG. Similarly, 10 measurements of Ag_2S (36 nm, HepaRG) and 10 measurements of AgO (50 nm, HepaRG). These measurements were excluded from statistical analysis as they did not meet the required variation of parameters and values. During the final observation of the dataset, 880 measurements were preserved and subjected to statistical analysis, which accounted for roughly 54% of the original dataset.

Statistical analysis

The statistical analysis was performed in R-Studio. Plots were generated with the ggplot2 package, and an interactive chart was created using the Plotly package. Multiple Ordinary Least Squares (OLS) regression was employed to analyze the data. This statistical technique estimates the coefficients of the linear relationship between the dependent variable and several independent variables by minimizing the sum of the squared differences between the observed and predicted values. In this paper, the relationship between cell viability and multiple regressors, such as dose, particle diameter, compound, and cell line was modelled.
For this analysis, two different model specifications were explored. The first was a Multiple OLS Model without interactions (Model 1), aimed at exploring the significance of certain variables and their effect on cell viability without considering any interactions. The second was a Multiple OLS Model with interactions (Model 2), which aimed to explore the significance of certain variables on cell viability while also considering interactions between some of the regressors.

The structural form of Model 1 was defined by Formula 1:

$$Cell\ Viability = \beta_0 + \beta_1(\log(Dose)) + \beta_2(Particle\ Diameter) + \beta_3(Compound) + \beta_4(Cell \quad (1)$$

Explanation of the Terms applicable for Formula 1:

- β_0: The intercept, representing cell viability when all independent variables are at their baseline values.
- β_1: The coefficient for the logarithmic exposure dose, showing how relative changes in the dose affect cell viability.
- β_2: The coefficient for particle diameter, indicating the effect of nanoparticle size on cell viability.
- β_3: The coefficient for the compound variable. Since *compound* is a categorical variable, β_3 denotes a vector of estimates (for particular compounds).
- β_4: The coefficient for the exposed cell line variable. Similarly to β_3, it is a vector of coefficients.
- ϵ: The error term, capturing variability not explained by the model.

The structural form of Model 2 was as follows:

$$Cell\,Viability = \beta_0 + \beta_1(\log(Dose)) + \beta_2(Particle\ Diameter) + \beta_3(Compound) + \beta_4(Cell\,lin \tag{2}$$

The difference between Model 1 (without interactions) and Model 2 (with interactions) was the addition of the interaction term between particle diameter and compounds. This enabled examination whether the effect of particle diameter was dependent on the compound or not.

Besides that, the sensitivity analyses were conducted, by sub-setting the final sample. First, the relationship between the independent variables and cell viability by compounds was explored. The goal was to investigate whether the significance (and the direction of the effect) of the dose and particle diameter (when applicable) stayed the same as in the full model. The structural equation for the Multiple OLS model by compounds was as follows:

$$Cell\,Viability_i = \beta_0 + \beta_1(\log(Dose_i)) + \beta_2(Particle\,Diameter_i) + \beta_3(Cell\,line_i) + \epsilon_i \tag{3}$$

where $i = 1, \ldots, N$.

Second, the sample was divided based on the cell lines. The structural equation for Multiple OLS model by cell lines was as follows:

$$Cell\,Viability_i = \beta_0 + \beta_1(\log(Dose_i)) + \beta_2(Particle\,Diameter_i) + \beta_3(Compounds_i) + \epsilon_i \tag{4}$$

where $i = 1, \ldots, N$.

Explanation of the Terms applicable for Formula 3 & Formula 4:

- β_0: The intercept, representing cell viability when all independent variables are at their baseline values.
- β_1: The coefficient for the logarithmic exposure dose, showing how relative changes in the dose affect cell viability.
- β_2: The coefficient for particle diameter, indicating the effect of nanoparticle size on cell viability.
- β_3: The coefficient for the cell line (formula 3) or compound variable (formula 4).
- ϵ_i: The error term, capturing variability not explained by the model.

Results

The refined database can be found in the supplementary documents of this paper, and the 880 measurements from seven datasets contained five cell lines and six compounds, and their combinations is displayed in Table 2.

Table 2: Compounds vs. Exposed Cell Line counts.

ExposedCells/ Compounds	A549	HCT116	HepaRG	HEPG2	RAW264.7
Ag	16	16	50	16	16
CeO_2	16	16	20	16	16
Cu	0	0	20	0	0
SiO_2	24	24	170	24	24
TiO_2	48	48	100	48	48
ZnO	16	16	40	16	16

Subsequently, the particle diameters ranged from 8 to 150 nm, and exposure doses ranged from 0.3 to 250 [µg/ml], as displayed in Table 3. Interestingly, ViableCellCount variable contained also positive values, which can be attributed to less toxic results compared to the control sample. Additionally, the exposure time was constant for all measurements – 24h.

Table 3: Summary statistics of continuous variables used in the model.

Statistic	Min	Mean	Median	Max	Std Dev
ExposureDosis	0.30	33.85	7.80	250.00	58.53
ParticleDiameter	8.00	39.86	20.00	150.00	43.57
ViableCellCount	-26.98	-2.02	-0.64	2.43	4.38
ExposureTime	24.00	24.00	24.00	24.00	0.00

Multiple OLS Model (with and without interactions)

The aim was to explore the significance of variables on cell viability variable (CVV), displayed in Table 4.

Table 4 : Results of model with and without interactions

	Dependent variable:	
	Viable Cell Count	
	Model 1	Model 2
	(1)	(2)
log(ExposureDosis)	-1.002^{***}	-1.002^{***}
	(0.063)	(0.063)
ParticleDiameter	0.011^{*}	0.003
	(0.006)	(0.036)
CompoundsCeO_2	3.094^{***}	4.610^{***}
	(0.482)	(1.740)
CompoundsCu	-5.821^{***}	-14.424^{***}
	(0.823)	(2.953)
CompoundsSiO_2	3.215^{***}	2.867^{***}
	(0.382)	(0.915)

$CompoundsTiO_2$	2.494***	2.417***
	(0.369)	(0.893)
CompoundsZnO	-2.369***	3.408
	(0.854)	(9.477)
ExposedCellHCT116	-0.823*	-0.823*
	(0.430)	(0.428)
ExposedCellHepaRG	-1.330***	-1.371***
	(0.365)	(0.367)
ExposedCellHEPG2	0.980**	0.980**
	(0.436)	(0.435)
ExposedCellRAW264.7	0.608	0.608
	(0.436)	(0.435)
$ParticleDiameter:CompoundsCeO_2$		-0.056
		(0.066)
ParticleDiameter:CompoundsCu		0.776***
		(0.250)
$ParticleDiameter:CompoundsSiO_2$		0.013
		(0.036)
$ParticleDiameter:CompoundsTiO_2$		0.003
		(0.037)
ParticleDiameter:CompoundsZnO		-0.033
		(0.074)
Constant	-1.556***	-1.362
	(0.444)	(0.891)
Observations	880	880
R^2	0.429	0.437
Adjusted R^2	0.422	0.426
Residual Std. Error	3.331 (df = 868)	3.317 (df = 863)
F Statistic	59.266*** (df = 11; 868)	41.842*** (df = 16; 863)
Note:		*p<0.1; **p<0.05; ***p<0.01

The first variable, *log(ExposureDosis)*, was negative and statistically significant for both models, having the same value (−1.002). A 100% increase in the exposure dose was associated with approximately a 1 percentage point decrease in CVV.

Results for compounds highlight the varying effect of each compound in comparison to the base compound, which was Ag. Compared to Ag, CeO_2, SiO_2, and TiO_2 had a positive and significant effect. On the other hand, Cu and ZnO resulted in a strong negative effect, indicating a substantial reduction in cell viability compared to Ag. Interestingly, results for ZnO nanoparticles in Model 1 are significant (compared to Ag, ZnO is associated with a 2.369 percentage point decrease in CVV), whereas in Model 2, which contains the interaction with particle diameter, the estimates are not significant.

For cell lines, the results were stable across both models in terms of significance and values. HCT116 and HepaRG cells displayed a negative effect on CVV and were statistically significant, while HEPG2 and RAW264.7 displayed a positive effect on CVV but lacked statistical significance, raising questions about the resilience (sensitivity) of specific cell lines to different compounds.

The variable *ParticleDiameter* had a statistically significant positive effect ($p < 0.05$) in the model without interactions. However, in the model with interactions, the effect of the diameter and compounds became more complex, as the direct effect was not immediately apparent.

We can derive the effect of diameter on CVV by taking the first derivative of CVV with respect to diameter (from Equation 2). The derivative is equal to $\beta_2 + \beta_5$*Compound, hence, the effect of diameter is now dependent on the compound. For Ag, the effect of diameter is exactly β_2, but this estimate is not significant. In contrast, diameter appears to play a role for Cu compounds. Increasing the diameter by 1 (or 10) nm is linked to a 0.776 (7.76) percentage point increase in CVV for Cu compared to Ag.

Analogically, we can analyze the difference between Ag and the other compounds. By taking the first derivative of CVV with respect to the compound, we get $\beta_3 + \beta_5$*Diameter. If the diameter was 0, there is no interaction effect and the effect of a compound on the outcome is exactly the respective estimate of β_3. However, the minimum value of diameter is 8 nm, meaning that the interaction term is taken into account. Particularly, the effect of Cu on CVV depends on the particle diameter as the interaction was significant. In other words, the effect of Cu on CVV can be computed as -14.424 + 0.776*diameter. As the particle diameter increases, the effect of Cu on CVV (compared to Ag) becomes less negative. If the diameter is 1 nm, the effect of Cu on CVV (compared to Ag and holding other variables constant) is -13.359. If the diameter is 10 nm, Cu would be associated with a 6.664 percentage point decrease in CVV compared to Ag.

Multiple OLS Model (by compounds)

The aim was to explore how the specific factors influence CVV separately for each compound (decreasing the sample size compared to the whole-sample regression) and whether these effects are consistent with the whole-sample results. The results by compound are displayed in Table 5 below.

Table 5: Results of Multiple OLS model by compounds

	Dependent variable:					
	Viable Cell Count					
	TiO_2	Ag	SiO_2	CeO_2	ZnO	Cu
	(1)	(2)	(3)	(4)	(5)	(6)
log(ExposureDosis)	-0.759^{***}	-1.697^{***}	-0.289^{***}	-0.122^{***}	-3.067^{***}	-3.136^{***}
	(0.086)	(0.162)	(0.048)	(0.040)	(0.273)	(0.547)
ParticleDiameter	0.005	-0.006	0.010^{***}	-0.053^{***}	-0.030	0.779^{**}
	(0.007)	(0.034)	(0.004)	(0.010)	(0.096)	(0.362)
ExposedCellHCT116	-0.525	2.079^{*}	-0.475	-0.202	-5.764^{***}	
	(0.517)	(1.082)	(0.422)	(0.221)	(1.723)	

ExposedCellHepaRG	-2.418***	2.519***	-1.853***	-0.736***	-1.972	
	(0.458)	(0.916)	(0.335)	(0.215)	(1.477)	
ExposedCellHEPG2	-0.227	5.956***	-1.047**	-0.506**	4.246**	
	(0.527)	(1.099)	(0.425)	(0.226)	(1.753)	
ExposedCellRAW264.7	0.032	4.392***	-1.339***	-0.860***	3.033*	
	(0.527)	(1.099)	(0.425)	(0.226)	(1.753)	
Constant	1.194***	-3.059***	0.794**	1.816***	6.386	-12.017**
	(0.411)	(1.046)	(0.313)	(0.323)	(13.918)	(4.329)
Observations	292	114	266	84	104	20
R^2	0.359	0.551	0.272	0.463	0.625	0.688
Adjusted R^2	0.345	0.526	0.255	0.421	0.602	0.651
Residual Std. Error	2.534 (df = 285)	3.061 (df = 107)	1.461 (df = 259)	0.625 (df = 77)	4.874 (df = 97)	4.852 (df = 17)
F Statistic	26.592*** (df = 6; 285)	21.909*** (df = 6; 107)	16.139*** (df = 6; 259)	11.062*** (df = 6; 77)	26.928*** (df = 6; 97)	18.741*** (df = 2; 17)
Note:				*p<0.1; **p<0.05; ***p<0.01		

The effect of *log(ExposureDosis)* was significant and negative (decreasing CVV) for all measurements, which was also by the previous results of models with/out interactions. The strongest effect was observed for ZnO (-3.067) and Cu (-3.136), while the weakest effect was noted for CeO_2 (-0.122) and SiO_2 (-0.289), which was primarily due to elemental characteristics. Regarding *ParticleDiameter*, there were contradictive results that varied across compounds. Only for three compounds, particle diameter was statistically significant; SiO_2 and Cu showed a positive relationship with particle size, while CeO_2 exhibited a negative effect. Positive effect indicated – the larger particles, the higher cell viability, the lower toxicity.

The results from these regressions indicate that HepaRG cells were particularly sensitive within most compounds (showing significant negative effects on viability) compared to the base A549 cells. Specifically, HepaRG cell lines were associated with a 2.418, 1.853, and 0.736 percentage point decrease in CVV for TiO_2, SiO_2, and CeO_2 (compared to A549 cell lines). This cell line was not significantly different from the base for ZnO, however, the HCT116 cell line was found to have a significantly negative effect on CVV (-5.764) in ZnO regression (compared to A549).

Interestingly, ZnO NPs showed a positive and significant effect on HEPG2 and RAW267.7 cells, and Ag NPs were positive and significant for all cell lines, with the highest positive effect of 5.796 for HEPG2. These results indicate that these cell lines might have had higher resistance to these NPs, or in the case of lower doses, ZnO NPs or Ag NPs could have acted as a nutrient, promoting cell growth or metabolic activity, which resulted in positive outcomes.

Multiple OLS Model (by cell lines)

The aim was to explore how the specific factors influence CVV separately for each cell line and whether these effects are consistent with the whole-sample results. The regression results are displayed in Table 6 below.

Table 6: Results of Multiple OLS model by cell lines

	Dependent variable:				
	Viable Cell Count				
	A549	HCT116	HepaRG	HEPG2	RAW264.7
	(1)	(2)	(3)	(4)	(5)
log(ExposureDosis)	-0.615***	-0.917***	-1.203***	-0.712***	-0.722***
	(0.118)	(0.148)	(0.105)	(0.087)	(0.102)
ParticleDiameter	-0.004	-0.001	0.014	-0.009	-0.016
	(0.013)	(0.017)	(0.009)	(0.010)	(0.011)
CompoundsCeO_2	5.556***	3.252***	4.136***	0.979*	2.231***
	(0.738)	(0.922)	(1.102)	(0.534)	(0.626)
CompoundsCu	N/A	N/A	-6.033***	N/A	N/A
	N/A	N/A	(1.110)	N/A	N/A
CompoundsSiO_2	5.905***	3.351***	3.207***	0.559	1.831***
	(0.669)	(0.836)	(0.691)	(0.484)	(0.567)
CompoundsTiO_2	5.632***	3.022***	1.915**	0.561	2.394***
	(0.598)	(0.748)	(0.726)	(0.433)	(0.508)
CompoundsZnO	4.091**	-4.183*	-4.162***	1.375	2.540
	(1.820)	(2.276)	(1.432)	(1.317)	(1.545)
Constant	-4.420***	-2.039***	-2.216***	0.591	-0.817
	(0.601)	(0.751)	(0.683)	(0.470)	(0.551)
Observations	120	120	400	120	120
R^2	0.544	0.584	0.427	0.390	0.421
Adjusted R^2	0.519	0.562	0.417	0.357	0.390
Residual Std. Error	2.072 (df = 113)	2.590 (df = 113)	4.164 (df = 392)	1.499 (df = 113)	1.758 (df = 113)
F Statistic	22.424*** (df = 6; 113)	26.488*** (df = 6; 113)	41.701*** (df = 7; 392)	12.022*** (df = 6; 113)	13.673*** (df = 6; 113)
Note:					$^{*}p<0.1$; $^{**}p<0.05$; $^{***}p<0.01$

Based on the results above, it is possible to see that the effect of *log(ExposureDosis)* is statistically significant and negative for all observations, indicates universal trend of lower CVV while increasing exposure dose. The strongest negative effect was observed for HepaRG cells (-1.203), while the weakest negative effect was displayed for A549 (-0.615), emphasizing unique sensitivities of cell lines for specific exposure doses. Particle diameter was not found to be significantly associated to CVV in any of the cell line regressions.

The significant positive coefficients for CeO_2, SiO_2, and TiO_2 indicate lower toxicity in comparison to Ag across various cell lines (A549, HCT116, HepaRG and RAW264.7).

The effect of ZnO on CVV, compared to Ag, was significantly different in A549, HCT116 and HEPARG regressions. For A549 cell line, the effect of ZnO NPs was positive (4.091), and for HCT116 (-4.183) and HepaRG (-4.162), the effect was negative. One of possible explanation could be that when interacting with A549, ZnO NPs could promote cellular mechanisms to maintain viability, or for HCT116 and HepaRG could induce cytotoxic effects more strongly, leading to lower CVV.

The last negative instance was observed for Cu NPs that were measured on HepaRG cell line, negatively affecting CVV by 6.033 percentage points. These results can be linked to either elemental characteristics or HepaRG liver cells being particularly vulnerable due to their high metabolic activity and susceptibility to oxidative damage and metal ion toxicity.

Discussion

The results above represent an exemplary attempt at a comprehensive statistical analysis of the data extracted from the NanoCommons database. Among a large number of available datasets, the attention is focused on six widely studied compounds in the form of NPs and their concentration effects on different cell lines while their size is also considered as the factor. The compounds selected from the database were zinc oxide (ZnO), copper (Cu), silver (Ag), titanium dioxide (TiO_2), silicon dioxide (SiO_2), and cerium dioxide (CeO_2), where each compound exhibited distinct toxicological behaviours associated with its properties, such as ion release, oxidative stress induction, or photocatalytic activity. The logical counterpart in consideration of any toxic agent effects is the living target, represented in vitro by the various cell lines derived from different human tissues having thus different susceptibility and response to the questioned toxic agent. So, to evaluate the effect of the choice of the cell lines, the CVV data subsets for HepG2, HepaRG, A549, RAW264.7, and HCT116 were investigated.

Prior to any other consideration, it should also be acknowledged that this study has several limitations. First, the analysis was conducted on the limited dataset, although being extracted from the dataset is as representative as possible. Moreover, factors such as shape, surface charge or modifications of NPs were not included in this study as they were not included in the original database. Next, the analysis is done in full awareness that the statistic evaluation enables us to find correlations without establishing causality relations.

The results of statistical analysis of different variables affecting CVV displayed wide range of findings in terms of interactions of various compounds, cell lines and NPs size. Their graphical visualisation in the form of the interactive 3D chart is available in supplementary data of the paper. In all models, the exposure dose was consistently displaying significant negative effect on CVV, which is supporting the universal trend of increased toxicity at higher doses, as can be seen in Figure 1.

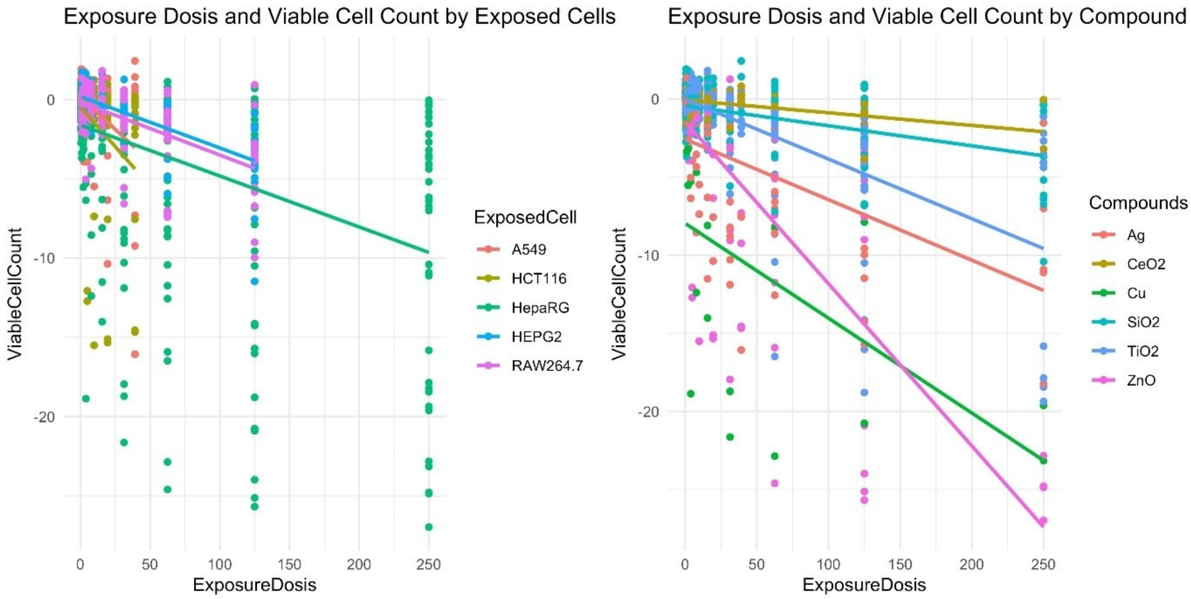


Figure 1: Effect of ExposureDosis on CVV from perspective of Exposed Cells (left) and Compounds (right)

Another principal view on the data shown in Figure 2 demonstrates the analysis of trends in CVV dependence on particle diameter.

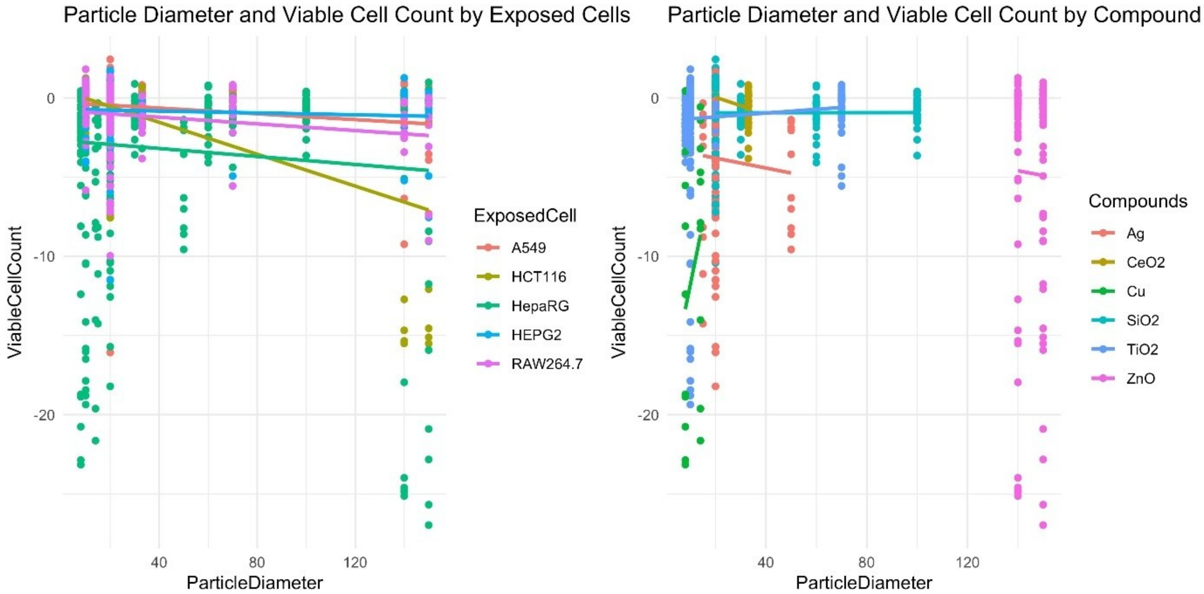


Figure 2: Effect of ParticleDiameter on CVV from perspective of Exposed Cells (left) and Compounds (right)

The particle size, although displaying negligible overall effect on CVV, showed compounds-specific impact in the model with interactions, particularly for Cu NPs where the increase in particle size was associated with positive effect on CVV (0.776), thus decreasing the total negative effect of Cu NPs on CVV. The results from model without interactions displayed that Cu and ZnO were associated with negative impact on CVV as expected (Ahamed et al., 2010; Fahmy and Cormier, 2009; Fukui et al., 2012; Naz et al., 2020; Song et al., 2010) while CeO_2, SiO_2 and TiO_2 were displaying positive effect on CVV, due to each compound characteristics – CeO_2 possibly behaving as a prooxidant (Pansambal et al., 2023; Singh et al., 2020), and TiO_2 possibly behaving relatively inert unless photoactivated (Arun et al., 2023; Baranowska-Wójcik et al., 2020; Ziental et al., 2020). In the case of SiO_2 there is no clear explanation why SiO_2 nanoparticles resulted to have positive effect on CVV. There are numerous studies displaying contradictory conclusions of SiO_2 toxicity, primarily focusing on the factor of surface modification (Marzaioli et al., 2014; Nayl et al., 2022; Yang et al., 2018), which in this study, had not been examined.

The multiple OLS model by compounds displayed that particle diameter was significant in three cases – for SiO_2, CeO_2 and Cu. The estimates were small for the first two, but for Cu NPs, the effect of particle size on CVV was significantly bigger compared to other compounds. For SiO_2 and CeO_2, the effect on all cell lines was negative, underlining the increased impact or higher sensitivity of selected cell lines within these two compounds.

The multiple OLS model by cell lines showed that compared to Ag, there was a positive relationship between the remaining compounds and CVV for A549 and RAW264.7 regressions. It can be due to Ag characteristics (Chairuangkitti et al., 2013; Giovanni et al., 2015) where specifically in these measurements decreasing CVV more than other nanoparticles or indicating a possible resistance of A549 and RAW264.7. For the latter, there is no literature or research supporting this assumption.
Next, only in three cases, the results surprisingly suggest a negative effect of a specific compound compared to Ag on CVV. In HEPARG regression, ZnO and Cu were found to decrease the CVV by 4.162 and 6.033 percentage points, respectively, compared to Ag. The next negative effect was also observed for ZnO NPs when exposed on HCT116 cells, decreasing CVV by 4.183. Since both ZnO and Cu can induce oxidative stress (Fukui et al., 2012; Song et al., 2010), the increased sensitivity of HCT116 and HepaRG cells could be attributed to their biological characteristics, with HepaRG liver cells being particularly vulnerable due to their high metabolic activity and susceptibility to oxidative damage and metal ion toxicity (Helvenstein et al., 2015; Kuhn et al., 2024; Voss et al., 2020).

In summary, the results have indicated a hierarchy within the four investigated factors influencing the toxicity scores of NPs in CVV as illustrated in Figure 3. First, it is the primary pair of factors, i.e. the composition and the choice of the counterpart cell line, followed by the concentration (or dose) which governs the overall manifestation of the NPs' toxicity in a significant decrease of CVV. The NPS size is of lesser importance, which means it governs the toxicity effect within the subset defined by the choice of the primary factor pair. This is contra-intuitive assuming the key role of size-related effects in nanomaterials, including the nanotoxicity as revealed by many experimental mechanistic studies (see (Macko et al., 2023) and references therein). It should be kept in mind that these results do not suggest that the size of NPs does not play any role in their toxicity mechanism (e.g. for Ag, TiO_2, and ZnO in this study), but the effect of various sizes has not been significantly manifested at testing conditions used in the original studies collected and integrated into the NanoCommons knowledge base.

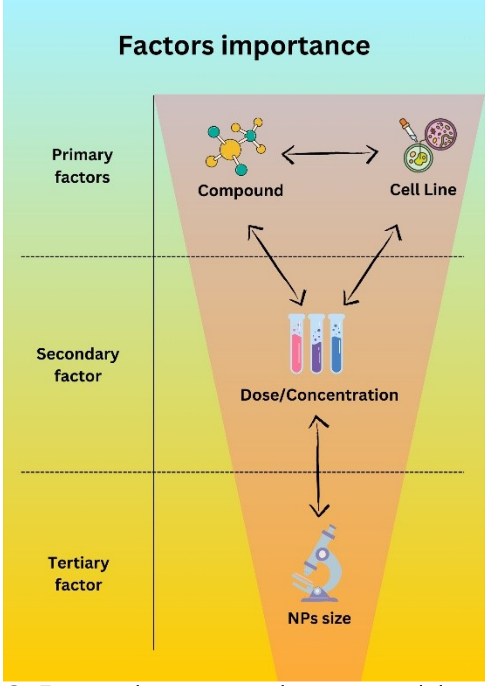


Figure 3: Factors importance in nanoparticles toxicity

Future research

When it comes to the future research of nanoparticles toxicity, and at the same time, enrichment of NanoCommons database, it would be beneficial to have more data on toxicity of Alumina NPs (Al_2O_3), which can release Al^{3+} ions, which are known to be neurotoxic and can interfere with cellular functions (Gao et al., 2022; Park et al., 2016, 2015; Strigul et al., 2009). To complete the picture of the most produced nanoparticles, Carbonaceous NPs should be subject to continuous research, as these also poses toxicological properties (Awasthi et al., 2024; Uo et al., 2011). Another addition to the database could be done on ultrafine dust particles (UFPs) collected at different locations. UFPs consist of particles below 100 nanometres, and pose various risks (cancer, neurological or respiratory health issues), at the same time, it is the most common nanoparticle in the environment (Hammond et al., 2022; Heinzerling et al., 2015; Kolling et al., 2011; Ophir et al., 2019). For the reasons above, addition and toxicological research on the abovementioned elements could enhance the relevance of the database and its metanalysis.

Conclusion

This study provides insights into the relationships between nanoparticle characteristics and cell viability. The effects of variables – NPs composition, cell line, dose and NPs size were explored via two main models – the first model was a Multiple OLS Model without interactions (Model 1), aimed at exploring the significance of certain variables and their effect on cell viability without considering any interactions. The second was a Multiple OLS Model with interactions (Model 2), which aimed to explore the significance of certain variables on cell viability while also considering interactions between some of the regressors. Subsequently, the sensitivity analyses were conducted by sub-setting the final sample. First, the relationship between the independent variables and cell viability by compounds was explored. Second, the sample was divided based on cell lines. The results displayed significant dose dependency on CVV across all models and confirmed the universal trend of increased toxicity at higher doses. The particle size had a negligible effect on CVV, but compound-specific impacts were observed. Overall, the results did not provide a clear trend on how other variables, such as cell lines or particle size, influence CVV but can serve as another supporting material in understanding nanoparticle toxicity. The factors can be ordered within a hierarchy of influence. The primary pair of factors is the composition and the choice of the counterpart cell line for testing, followed by the secondary factor of concentration (or dose), which governs the overall manifestation of the NPs' toxicity in a significant decrease of CVV. The tertiary factor of NPs size is manifested of lesser importance, which might be rather due to the testing conditions than due to the irrelevance of the size at all.

This study also demonstrated the usefulness of data collections present in the NanoCommons database and indicates its future potential for data extraction in the field of nanotoxicity. Moreover, the amount of gathered FAIR (Findable, Accessible, Interoperate, and Reusable) data surpasses the magnitude of any individual experimental study. The observed correlations may be a stepping stone for targeting new studies and developing more sensitive nanotoxicity testing methods to overbridge the gap between mechanistic and statistical screening studies.

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