

Meta-analysis of in-vitro cytotoxicity evaluation studies of zinc oxide nanoparticles: Paving way for safer innovations

Anaida Kad^a, Archit Pundir^a, Shailendra Kumar Arya^a, Sanjeev Puri^a, Madhu Khatri^{a,b,*}

^a Department of Biotechnology, University Institute of Engineering and Technology, Panjab University, Sector-25, Chandigarh 160014, India

^b Wellcome trust/DBT IA Early Career Fellow Panjab University, Chandigarh 160014, India

ARTICLE INFO

Editor: Dr. P Jennings

Keywords:

Zinc oxide nanoparticles
Cell viability assays
Cytotoxicity
meta-analysis
Systematic review
Decision trees
Forest plots

ABSTRACT

Nano-based products have shown their daunting presence in several sectors. Among them, Zinc Oxide (ZnO) nanoparticles wangled the reputation of providing “next-generation solutions” and are being utilized in plethora of products. Their widespread application has led to increased exposure of these particles, raising concerns regarding toxicological repercussions to the human health and environment. The diversity, complexity, and heterogeneity in the available literature, along with correlation of befitting attributes, makes it challenging to develop one systematic framework to predict this toxicity. The present study aims at developing predictive modelling framework to tap the prospective features responsible for causing cytotoxicity in-vitro on exposure to ZnO nanoparticles. Rigorous approach was used to mine the information from complete body of evidence published to date. The attributes, features and experimental conditions were systematically extracted to unmask the effect of varied features. 1240 data points from 76 publications were obtained, containing 14 qualitative and quantitative attributes, including physiochemical properties of nanoparticles, cell culture and experimental parameters to perform meta-analysis. For the first time, the efforts were made to investigate the degree of significance of attributes accountable for causing cytotoxicity on exposure to ZnO nanoparticles. We show that in-vitro cytotoxicity is closely related with dose concentration of nanoparticles, followed by exposure time, disease state of the cell line and size of these nanoparticles among other attributes.

1. Introduction

Zinc oxide (ZnO) is one of the first nanoparticles to be approved by the FDA and gain consumer acceptance at wide scale, especially in skincare and cosmetics (Chauhan et al., 2021; Holmes et al., 2020a). A wide band gap of 3.36 eV, high luminescent efficiency, and a large exciton binding energy of 60 meV, along with distinct intrinsic properties like semi-conducting nature, chemical sensing, electrical conductivity, optical, and piezoelectric of ZnO triggered intense research with regard to its applications in various fields (Król et al., 2017). The years have witnessed ZnO gaining competence as a key industrial constituent, progressing towards a remarkable rise in diverse applications, including nano-optical, nano-electrical, diagnostic, bioimaging, biosensor, and other similar machines. Additionally, it has also shown potential in food

packaging, medicines, cosmetics, agriculture, textiles, and everyday products as shown in Fig. 1 (Carmo et al., 2022; El-Ansary et al., 2021; Husain et al., 2022; Kaur et al., 2020; Khan et al., 2022; Liam Critchley, 2020; Prajitha et al., 2019; Sharma et al., 2019; Singh et al., 2021a).

During the viselike grip of SARS-Cov-2 pandemic, zinc has also proven to play a pivotal role in preventing viral infection (Joachimik, 2021; Pal et al., 2020; Skalny et al., 2020). While considering the business implications of COVID-19 pandemic and its subsequent economic crisis, the market for nanomaterials is expected to grow at 11.3% CAGR from 2020 to 2027 (“Global Nanotechnology Industry,” n.d.). Similarly, the ZnO nanoparticles market, which was valued at \$4.4 billion in 2019, is expected to reach \$5.7 billion by 2024, registering a CAGR of 5.4% during the forecast period of 2019–2024 (“Zinc Oxide Market Global Forecast to 2024 | MarketsandMarkets,” n.d.).

Abbreviations: ZnO, Zinc Oxide; FDA, Food and Drug Administration; CAGR, Compound Annual Growth Rate; ROS, Reactive Oxygen Species; QSAR, Quantitative Structure Activity Relationship; OECD, Organization for Economic Co-operation and Development; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; NP, Nanoparticle; SMD, Standard Mean Difference; CI, Class Interval.

* Corresponding author at: Department of Biotechnology, University Institute of Engineering and Technology, Panjab University, Sector-25, Chandigarh 160014, India.

E-mail address: madhuk@pu.ac.in (M. Khatri).

<https://doi.org/10.1016/j.tiv.2022.105418>

Received 23 March 2022; Received in revised form 3 June 2022; Accepted 14 June 2022

Available online 17 June 2022

0887-2333/© 2022 Published by Elsevier Ltd.

In spite of this, concerns have been raised about the safety of ZnO nanoparticles over the past two decades due to insufficient knowledge about their effects on humans, animals, and the environment. The main entry routes involved are inhalation through the respiratory tract, transudation/adsorption through the skin, and ingestion through the digestive tract, the same have been highlighted in Fig. 2 (Chen et al., 2022; Singh et al., 2021b). It is important to consider experimental and exposure parameters in toxicity studies in addition to the ZnO nanoparticles' unique physicochemical characteristics, which vary with their size, charge, surface modification, zeta potential, and shape. Most of the current literature on nanoparticle toxicity is fragmented by studies that only examine a few cross-sectional attributes in a given experimental system. Moreover, it is often very difficult to attain definitive conclusions regarding the toxicity posed by these nanoparticles because the risk evaluation is also influenced by the type of methodological approaches, along with different in vitro and in vivo models (Czyżowska and Barbasz, 2020). The toxicity is generally measured by estimating the extent of morphological and molecular damages, such as cell shrinkage, cell membrane damage, ROS generation, oxidative stress, up regulation of shock proteins, changed permeability of cell membrane, cytoskeleton components damage, nuclear condensation, formation of apoptotic bodies, DNA damage, changed concentration of lysosomal hydrolases, mitochondrial dysfunction, cytoplasm impairment, and others (Al-Ali et al., 2022; Attarilar et al., 2020; Medici et al., 2021). Therefore, as the literature is flooded with published data indicating applications and toxicity of nanoparticles in equal proportion, the scientific community seems divided on the aspect of safety of these nanoparticles. This vast testing landscape leads to variability and uncertainties, with no common consensus within the ZnO nanotoxicology literature; therefore, limiting the generalization of independent experiments' findings. Moreover, it cannot be ruled out that more than one physiochemical property plays a role in either enhancing or suppressing the toxicity. Nevertheless, the inter-linkages and correlation of befitting attributes along with their defined hierarchy, which may be responsible for causing toxicity still needs to be explored, analyzed, and utilized for minimizing the toxicological impact of ZnO nanoparticles.

So far, a systematic approach to investigate the potential risks of ZnO

nanoparticles that attempt to produce a generalized model is lacking. The *in-silico* methods either use qualitative classification-based models (banding models) or quantitative structure activity relationships (QSARs) for predicting the safety profiles of these nanoparticles (Afanitis et al., 2020). The sole focus of these models is to investigate and draw conclusions based upon limited dataset from few individual studies rather than considering the "entire" body of published evidence. Fortunately, meta-analysis, or data mining using knowledge extraction from previously published literature, has the potential to reveal hidden relationships that were not evident in the original investigations. Meta-analysis is the most essential component of systematic review, which gives statistical significance to the collected literature. A systematic review collects all possible studies related to a given topic and design, with distinct inclusion and exclusion criteria, reviews and analyze their results (Furxhi et al., 2020). There are various guidelines issued for reporting meta-analysis of random control trials, such as Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and OECD guidelines, to standardize and improve the quality of systematic reviews and meta-analysis. To date, attempts for meta-analysis of published data to test toxicity in nanoparticles have been limited to carbon nanotubes, quantum dots, titanium oxide nanoparticles, and generalized study for all nanoparticles (Gernand and Casman, 2014; Labouta et al., 2019; Ling et al., 2021; Oh et al., 2016). Furthermore, the studies seldom take into account the publication biases that may be present, owing to the selection of only published data or restrictive inclusion/exclusion criteria. Moreover, the wide spectrum of the body of evidence often contains partial/missing information across different experimental settings that leads to high data complexity. Therefore, the present study attempts at integrative analysis of the complete body of evidence for in vitro cytotoxicity caused by ZnO nanoparticles using extensive literature mining for database creation, data extraction, and classification on the basis of different parameters, followed by meta-analysis using forest plots, 3-D scatter plots, and decision trees. The study aims to reveal the cytotoxicity potential of ZnO nanoparticles to provide a reference for hazard assessment.

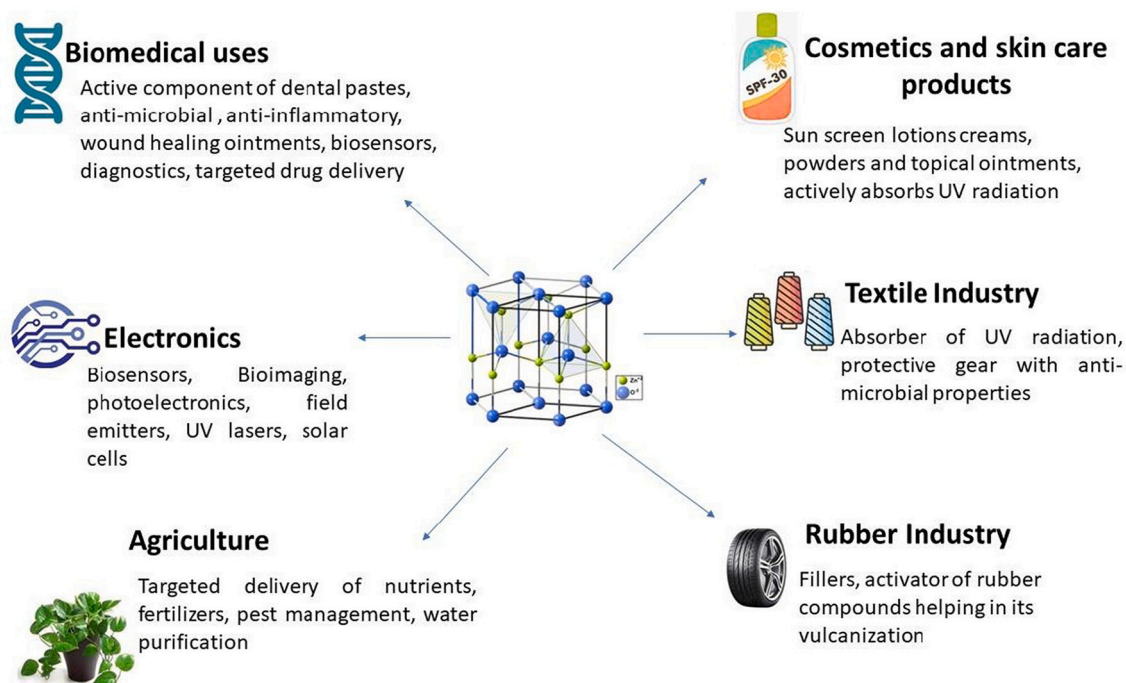


Fig. 1. Applications of ZnO nanoparticles in different sectors/disciplines.

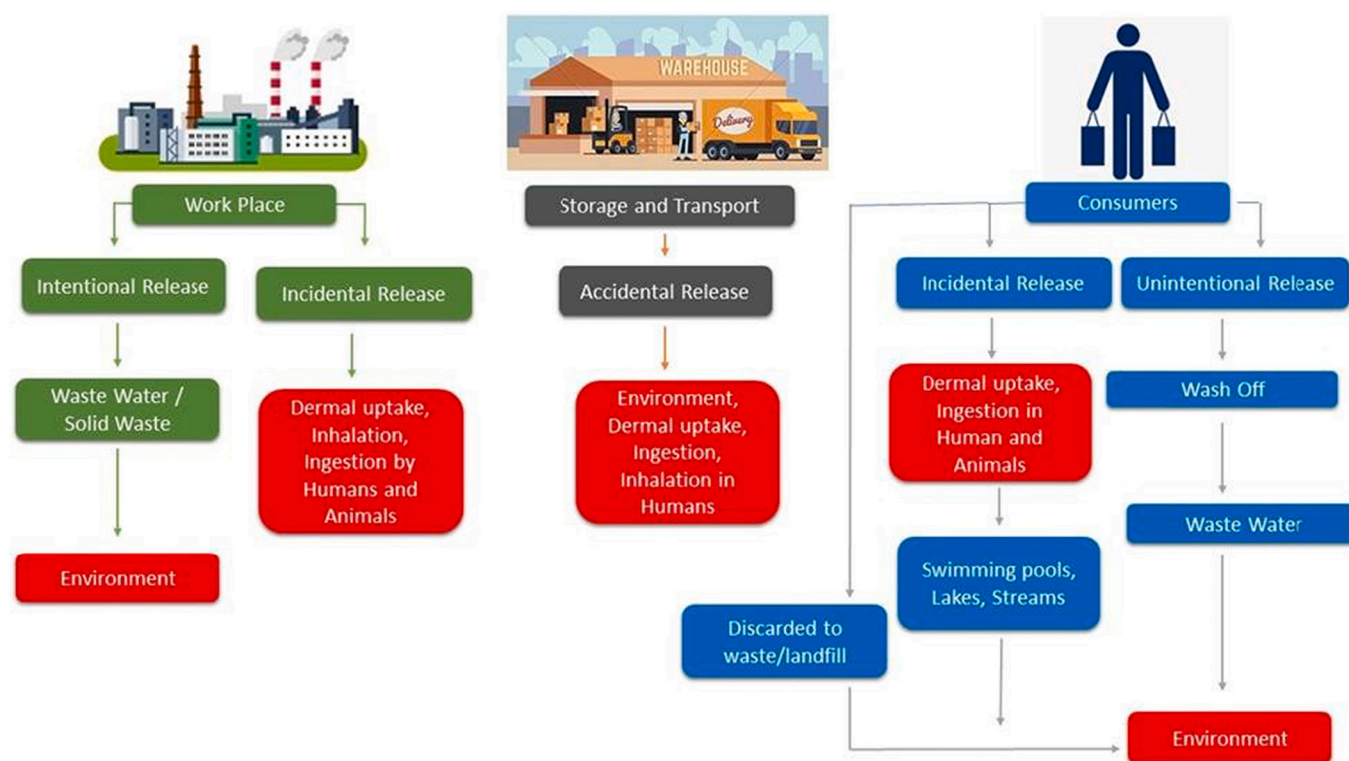


Fig. 2. Schematic representation indicating sites of potential exposure to ZnO nanoparticles destined for commercial use, encompassing their manufacture in the workplace, transport to markets, and consumption, followed by disposal by the consumers. The impact on humans, animals and the environment has been highlighted using red boxes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

We followed the defined workflow as depicted in Fig. 3. The first step consisted of extracting and compiling literature of ZnO toxicity, incorporating cytotoxicity assay and cell viability, together with their physicochemical properties and experimental conditions. Categorical or discrete attributes provided non-continuous variable information (for example: cell origin, shape of the particle, cell-line, etc.). Compiled data was pre-processed and normalized to attain standard units of measurement including exposure time, various concentration metrics and measure of cell viability. This was followed by defining and setting the ranges while categorically removing the excessive attributes of different categories. Based upon the finalized literature, 3-D scatter plots were made to detect the combined effect of two factors which individually contributed in causing toxicity posed by ZnO nanoparticles. Three properties which were repeated throughout the literature and held responsible for causing toxicity were tested in different permutations to attain 3-D plots. Based on the pre-processed ZnO toxicity data, Forest plots were developed for analyzing cell viability. The publication bias

was assessed using Fail-safe N method and Inverse Funnel Plot. Furthermore, decision trees were formed for sub-group analysis and identification of hierarchy of attributes responsible for causing toxicity.

2.1. Data mining through literature search

Various search engines were used with defined set of keywords and the papers were explored for potential research articles. PubMed, and Scopus were searched using “Zinc oxide nanoparticles or ZnO NP” and “toxicity or cytotoxicity or adverse effects or risks or cell viability.” Same keywords were used for Google scholar and the first twenty pages for every search were considered. All studies published from January 1, 2010, to January 31, 2022, in English were included.

2.2. Inclusion and exclusion criteria

The articles were then screened based upon defined inclusion and exclusion criteria. Studies, which at least incorporated the following features were selected for further investigation: (1) the characteristics of

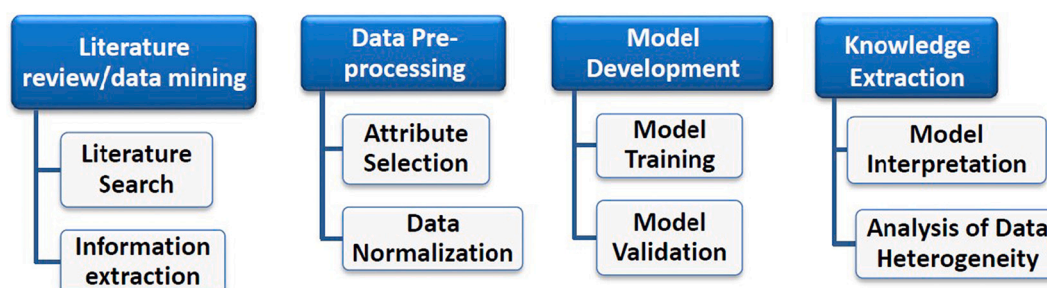


Fig. 3. Schematic representation of the procedure adopted for Meta-analysis.

ZnO nanoparticles (origin, size, shape, surface charge), (2) the characteristics of cell-line used for toxicity analysis (name, cell-type, tissue-organ of origin, species of origin, disease type), (3) cell viability assays using metabolism reductase evaluated by tetrazolium salts like MTT, MTS, WST-1, and CCK-8, resazurin based assays like Alamar blue, and dye based assays like Trypan Blue, SRB, and NRU, (4) the experiments which included negative and positive controls; and (5) duplicate or independent experiments present.

The stringently followed exclusion criteria kept the following studies out of the purview of meta-analysis: (1) reviews articles, expert opinions, book chapters, and meeting articles, (2) reports lacking numerical data for in vitro cytotoxicity caused by ZnO nanoparticles, (3) articles containing in vivo cytotoxicity studies caused by ZnO nanoparticles, (4) studies considering nanocomposites, and (5) research using 2-D and 3-D cell cultures.

The studies were separately screened for all the required information by two researchers independently.

2.3. Data extraction

Initially, the data was extracted based on three parameters, which are the title of the article, the authors, and the publication year to eliminate the duplicates and articles in languages other than English. Once the data was screened and abstracts analyzed, the articles were studied to retrieve the information for fifteen attributes or labels that were considered, to check the degree of influence, each of these attributes had on the label used for cytotoxicity. The features extracted include, ZnO nanoparticle physiochemical attributes like source, size, shape, zeta potential, and surface charge, the cell line attributes, such as species of origin, tissue from which it has been derived, anatomy of the cells, name of the cell line, disease state, passage or primary stage of the cells, and procedure/protocol specific features like cytotoxicity assay used to discern cell viability, exposure time, dose concentration, cell concentration per well, sample size of experimental group, and sample size of control group. Some of the attributes were harmonized for consistent units of measurement. The outcome index results were extracted in the form of cell viability measured as Mean $\pm$ standard deviation (SD) using WebPlotDigitizer (Ankit Rohatgi, n.d.).

2.4. 3-D scatter plots

To examine the association between cytotoxicity caused by ZnO nanoparticles and the extracted attributes, three features were tested in different combinations to create 3-D scatter plots. These included ZnO size, dose concentration, and exposure time of the cells to nanoparticles.

2.5. Statistical analysis for forest plots

In this meta-analysis, standard mean difference (SMD) was used as an effect size indicator to analyze each included study. SMD expresses the size of the intervention effect in each study relative to the variability observed in that study.

$$SMD = \frac{\text{Difference in mean outcome between the groups}}{\text{Standard deviation of outcome among participants}}$$

SMD = 0 indicates no difference between the exposure and control groups, whereas SMD < 0 indicates that the exposure group can cause higher cytotoxicity than the control. Statistical heterogeneity was analyzed by I-squared (I^2) statistic. The I^2 values of $\leq 50\%$ were considered to be homogeneous, and the fixed-effect model was used to calculate the SMD and its 95% CI. Otherwise, the random-effect model was used to combine the effect size indicators. Publication bias was evaluated using the visual funnel plot, Fail-Safe N calculation, Begg and Mazumdar.

Sensitivity analysis was performed by gradually eliminating individual studies to estimate whether one study affects the results. The

experiments were performed using Jamovi Version 1.6.16. All tests were 2-tailed and $P < 0.05$ was considered statistically significant (Software, 2020; Team et al., 2013; Van Houwelingen et al., 1993; Viechtbauer, 2010).

2.6. Decision tree model: Extent of toxicity posed by each attribute

Another machine learning approach, called decision tree was used to identify the extent of each feature of ZnO nanoparticles/model cell-lines/experimental conditions responsible for causing cytotoxicity. Each of these measurable attributes or characteristics being observed was termed as a 'feature'. Each algorithm took an input of complete dataset which was divided into 70–30 ratio, where 70% formed the training dataset while 30% was used as the test dataset. The program produced a 'classifier' that learnt to predict a 'label' based on patterns found in the training dataset, a dataset that typically involves many features. In this case, the 'label' was low cell viability (for $< 50\%$) or high cell viability (for $\geq 50\%$). The classifier could then take a new 'instance' (corresponding to a vector of available feature information, but not the "% cell viability" label) and return a predicted label for the instance in question. For this purpose, decision tree analysis took into consideration nine attributes or features, which were source of ZnO nanoparticles (commercial vs. in-house synthesized), size, shape and charge of nanoparticles, cell type (normal vs. cancer cells), cell source (animal vs. human), cell-origin (primary vs. cell-line), exposure time, and ZnO treatment concentration.

The Gini Index was used to identify the value of each attribute and the extent of its effect on the overall toxicity. The Gini Index measures the probability or degree of a particular variable being wrongly classified when it is randomly chosen (Tangirala, 2020). Hence, lower the value, better is the attribute classified. Initially, one decision tree was created for all the samples. Later, the data sets were divided into eight sub-parts based upon the cytotoxicity assays used for measuring cell viability percentage. Therefore, eight decision trees were made and the accuracy of each was analyzed.

3. Results and discussion

3.1. Literature mining for data selection

Through exhaustive data mining, a total of 2491 records were obtained using PubMed, Scopus, and Google Scholar. A few of these were excluded because of duplicates and articles in language other than English. The titles and abstracts of the rest 2043 articles were scanned to eliminate the records which were expert opinions, review articles, book chapters, used in vivo experiments for toxicity assessment, ZnO present as nanocomposites, 2-D or 3-D cell cultures. This extensive literature analysis identified 164 publications which were downloaded and scanned as per inclusion and exclusion criteria. The articles lacking replicates, independent experiments, and essential quantitative data were subsequently eliminated. After reading and analyzing the complete publications, 76 records were selected for meta-analysis (Abbasi-Oshaghi et al., 2018; Ahmad et al., 2020; Alarifi et al., 2013; Almutairi et al., 2020; Baek et al., 2011; Bai et al., 2017; Bandeira et al., 2020; Cao et al., 2020, 2015; Chen et al., 2015, 2019; Chevallet et al., 2016; Danielsen et al., 2015; De Angelis et al., 2013; Dekkers et al., 2018; Demir et al., 2014; Fouda et al., 2018; Fu et al., 2017; Fukui et al., 2012; Gao et al., 2016; Giovanni et al., 2015; Gong et al., 2017; Gopala Krishna et al., 2017; Guo et al., 2013; Han et al., 2016; He et al., 2017; Heng et al., 2010; Ho et al., 2017; Holmes et al., 2020b; Ickrath et al., 2017; Jain et al., 2019; Jeong et al., 2013; Johnson et al., 2015; Kang et al., 2013; Kim et al., 2013, 2014; Kononenko et al., 2017; Lai et al., 2015; Lee et al., 2014; Li et al., 2012, 2020; Liang et al., 2018; Liu et al., 2016, 2020; Mantecchia et al., 2017; Miri et al., 2020; Moratin et al., 2018; Namvar et al., 2015; Ng et al., 2017; Pati et al., 2016; Pinho et al., 2020; Poier et al., 2020; Qin et al., 2020; Safar et al., 2019; Sahu et al., 2014;

Saptarshi et al., 2015; Schneider et al., 2017; Shalini et al., 2018; Sharma et al., 2018, 2017, 2012; Song et al., 2019; Sudhakaran et al., 2019; Titma et al., 2016; Tolliver et al., 2020; Tripathy et al., 2014; Vallabani et al., 2019; Wang et al., 2015; Wang et al., 2018a; Wang et al., 2020a; Wang et al., 2018b; Wang et al., 2020b; Wu et al., 2018; Yan et al., 2020, 2014; Zhang et al., 2017). The results obtained at each stage of data extraction are summarized in Fig. 4.

3.2. Literature screening for information extraction

Literature data-mining for ZnO cell viability data, attributes

representing ZnO nanoparticles physicochemical properties, type of cytotoxicity assay, nature of the cells used and experimental conditions yielded 1240 cell viability percentage data samples as shown in Online Resource 1. Each study contributed equally with an exception of one publication. Therefore, the data was not over fitted or dominated by specific study. The summary of all the attributes extracted from the complete data set of 1240 data samples is mentioned in Fig. 5. On direct scrutiny, the compiled data shows no observable trend in cell viability as a function of size, exposure time and concentration of ZnO nanoparticles administered to cell cultures. This indicated the need for in-depth analysis of the compiled data to answer the overall question of

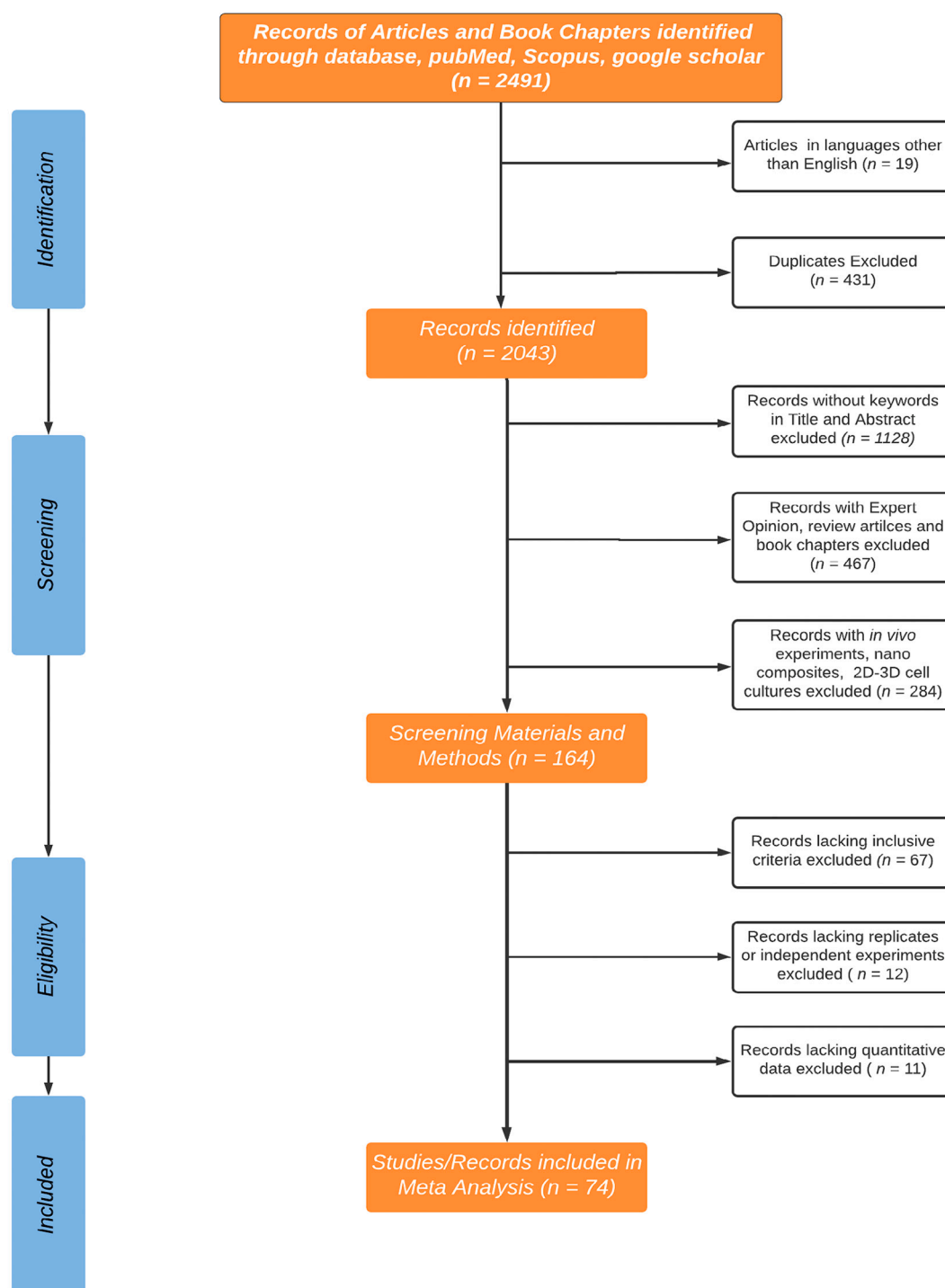


Fig. 4. Flow diagram of the process followed in the literature search.

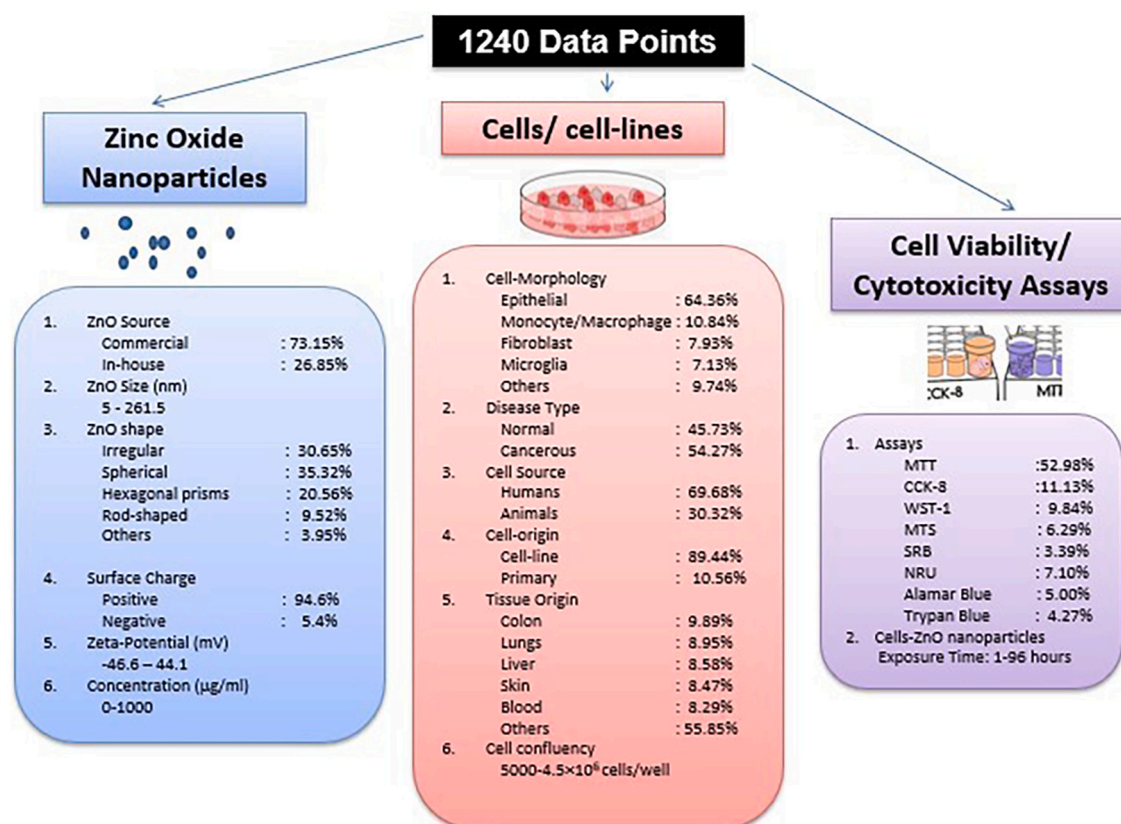


Fig. 5. Summary of attributes/features in 1240 data points extracted from literature. A total of 14 features were considered including ZnO nanoparticles related features (source, size, shape, surface charge, zeta potential, and concentration), cell-lines related attributes (morphology, disease type, source, origin, tissue, and confluency) along with assay type and exposure time.

toxicity posed by these nanoparticles and the attributes that need more scrutiny to enhance the safety profile of ZnO nanoparticles.

3.3. 3-D scatter plots

In order to permeate a co-relation between three parameters of ZnO nanoparticles always held accountable for causing toxicity in cell lines, 3-D plots were generated. The plots were created among the following features: (1) size(nm), dose concentration (µg/ml), and cell viability percentage; (2) size (nm), exposure time (h), and cell viability percentage; and (3) exposure time (h), dose concentration (µg/ml), and cell viability percentage. The obtained results depicted in Fig. 6 (a-c), indicated clear trend to decipher the causative agent of cytotoxicity; consequently, it became imperative to develop a model for further analysis.

3.4. Statistical analysis for forest plots

Upon reviewing the collected literature, it was observed that eight cytotoxicity assays were used primarily for assessing the cell viability of cells exposed to ZnO nanoparticles. For ease of meta-analysis, the 1240 data points were divided into 8 categories on the basis of these cell viability assays. Owing to the large number of data samples and the number of the assays, the forest plots of two assays have been discussed here. The results showed high similarity, that is, the outcomes obtained were relatively same. Therefore, the two tests were selected at random.

3.4.1. Forest plot for WST-8 assay

A total of $k = 138$ data samples were included for meta-analysis under this category. The observed standardized mean difference (SMD) ranged from -329.8702 to 5.1261 , with the majority of estimates

being negative (92%). This indicated that the exposure group caused higher cytotoxicity than the control. The estimated average SMD based on the random-effects model was -8.5305 (95% CI: -10.0582 to -7.0029), indicating higher cytotoxicity as the overall outcome for experiments exposed to ZnO nanoparticles than the control. This is detailed in Fig. 7, representing the forest plot for WST-8 assay. Therefore, the average outcome differed significantly from zero ($z = -10.9445$). According to the Q-test, the true outcomes appear to be heterogeneous ($Q(137) = 998.3915$, $\text{Tau}^2 = 68.4118$, $I^2 = 97.2871\%$). A 95% prediction interval for the true outcomes was -24.8135 to 7.7524 . An examination of the studentized residuals revealed that none of the studies had a value larger than ± 3.5661 and hence, there was no indication of outliers in the context of this model.

3.4.2. Forest plot for trypan blue assay

A total of $k = 53$ studies were included in the meta-analysis for Trypan Blue cell viability assay. The observed SMD ranged from -254.1772 to 1.9754 , with the majority of estimates being negative (91%). This indicated that the exposure group caused higher cytotoxicity than the control. The estimated average SMD based on the random-effects model was -4.7991 (95% CI: -6.1611 to -3.4371), indicating higher cytotoxicity as the overall outcome for experiments exposed to ZnO than the control. Therefore, the average outcome differed significantly from zero ($z = -6.9061$). According to the Q-test, the true outcomes appeared to be heterogeneous ($Q(52) = 295.6545$, $\text{Tau}^2 = 18.3056$, $I^2 = 93.4041\%$). The detailed forest plot is added as Fig. 8. A 95% prediction interval for the true outcomes was -13.2947 to 3.6965 . An examination of the studentized residuals revealed that two studies (Giovanni2015.4; Giovanni2015.8) had values larger than ± 3.3069 and may be potential outliers in the context of this model. These were eliminated and the tests were repeated to eliminate the publication bias.

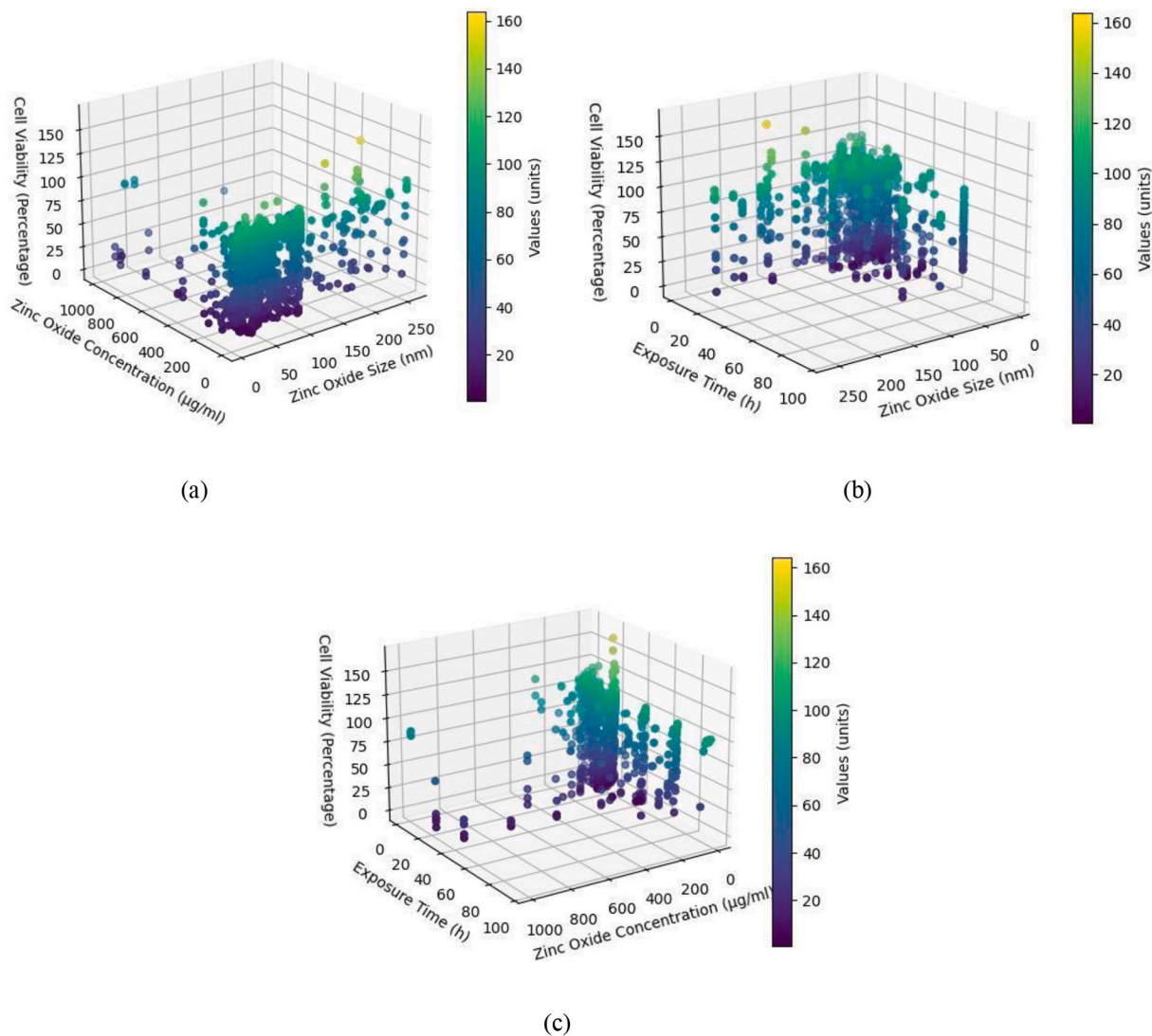


Fig. 6. (a) 3-D Scatter Plot for size of ZnO nanoparticles, its concentration and cell viability measured in percentage (b) 3-D Scatter Plot for size of ZnO nanoparticles, exposure time and cell viability measured in percentage (c) 3-D Scatter Plot for concentration of ZnO nanoparticles, exposure time and cell viability measured in percentage.

According to the Cook's distances, none of the studies could be considered to be overly influential.

According to these forest plots, and the cumulative result of the other six statical assays, meta-analysis results indicated that ZnO nanoparticles exposure exhibited high levels of toxicity leading to low cell viability as compared to the control group. The extent of influence of the individual attributes responsible for causing cytotoxicity was measured through Decision Trees.

3.5. Decision tree models to determine the attributes causing cytotoxicity

Though the toxicity of ZnO nanoparticles was established using the Forest plots, significant attributes accountable for causing cytotoxicity and their extent of contribution was yet to be determined. For this purpose, 9 decision trees were created. In all, nine attributes were tested, including source (commercial vs. in-house), size, shape, surface charge (positive vs. negative) of ZnO nanoparticles, cell species of cell-line source (animal vs. human), disease state of cell-line (normal vs. cancerous), cell culture stage (primary vs. cell-line), dose concentration and exposure time to ZnO nanoparticles.

The 1240 data samples were altogether used in the first decision tree to check the effect of distinctive features on the label, that is, cell

viability. As a result of getting highly complex decision tree, with 13 levels of hierarchical division and multiple attributes having relatively similar Gini Index, distinct and concrete results needed further classification. However, the dose concentration of ZnO nanoparticles formed the root node, therefore, emerging as the most important determinant of cytotoxicity caused by the exposure to the nanoparticles under in vitro conditions. Although four of the eight decision trees have been discussed for quick reference, the inference was drawn on the basis of cumulative analysis of all the decision trees.

3.5.1. Decision tree for cytotoxicity analysis using Alamar blue assay

The entire decision tree was visualized in top-down or hierarchical format. Leaf nodes that had no branches arising out of them with 5 or lower instances were removed to optimize the results and minimize the noise. The model gave predictable results, identifying four features responsible for causing toxicity, as shown in Fig. 9. The first rank parameter and the most important determinant of cell viability was dose concentration of the nanoparticles. Other three eminent attributes as per the model were disease state of the experimental cells, exposure time of the experiment and the size of the nanoparticles, to be considered in descending order. The model achieved an accuracy of 94.73%.

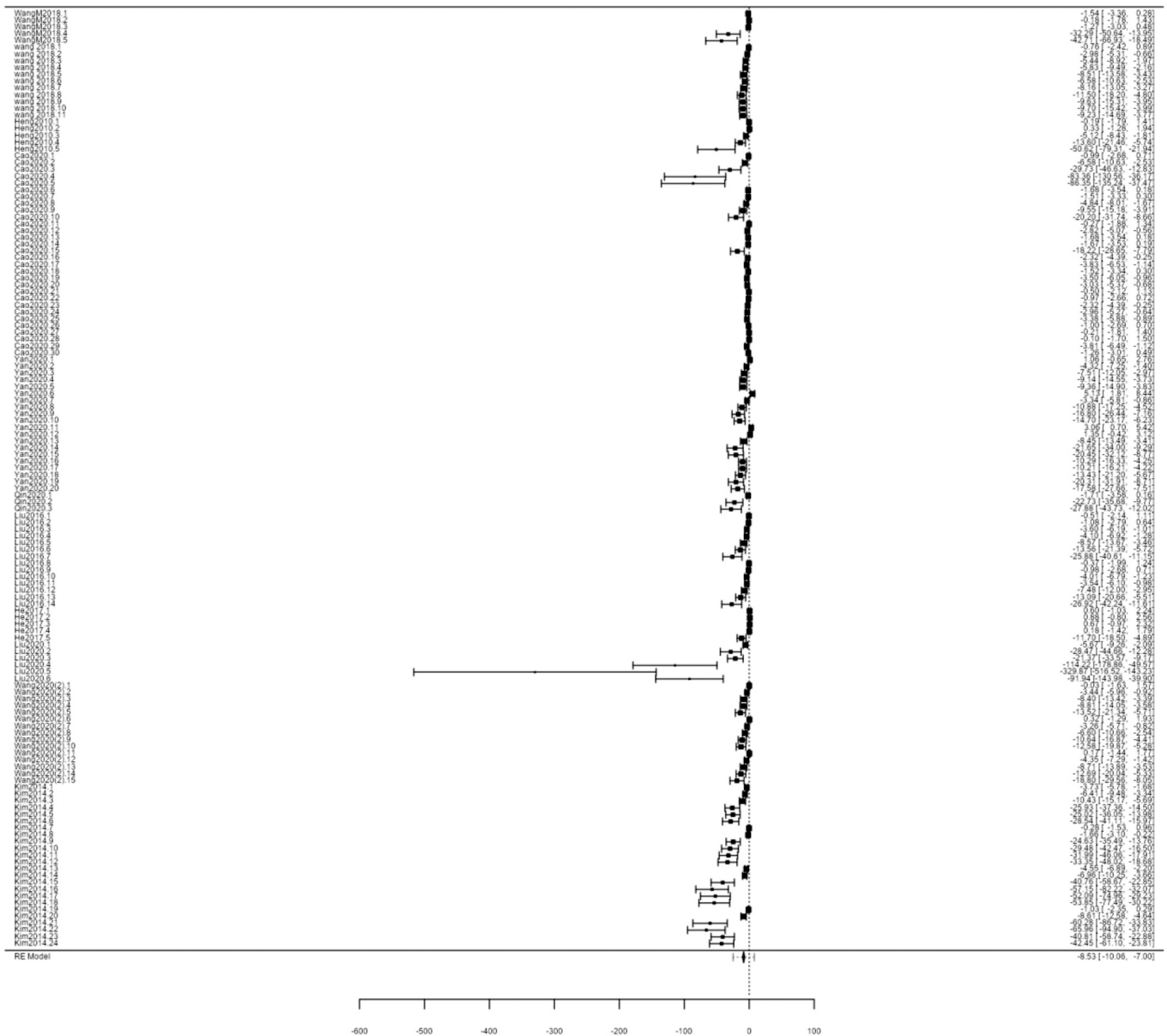


Fig. 7. Forest Plot meta-analysis of the cytotoxic effects of ZnO nanoparticles vs. control using WST-8 Cell Viability Assay.

3.5.2. Decision tree for cytotoxicity analysis using trypan blue assay

Considering the moderate number of data samples from literature, which used the Trypan Blue assay to evaluate the cytotoxicity of ZnO nanoparticles, this predictive model was not expected to perform at such a high level of accuracy. But contrary to the assumption, the model achieved 93.75% of accuracy, identifying diseased state of the test cells, dose concentration of ZnO nanoparticles, exposure time and charge of the nanoparticles as essential attributes contributing in deciding the cytotoxicity posed by these nano-sized ZnO to the cell cultures, as shown in Fig. 10.

3.5.3. Decision tree for cytotoxicity analysis using NRU assay

For data points utilizing NRU cell viability assay to check the cytotoxic effects of ZnO nanoparticles, the decision tree model exhibited 88.88% accuracy. The model had highly complex decision tree, divided into 7 hierarchical stages, and identified ZnO dose concentration added to cell culture, exposure time, ZnO nanoparticles' size, charge, source, passage stage, and species of origin for cell line as the important attributes responsible for causing cytotoxicity, mentioned in decreasing

order.

3.5.4. Decision tree for cytotoxicity analysis using WST-8 assay

As it is well known, WST-1 and WST-8 are similar cytotoxicity assays, where WST-8 is an improvisation over the pre-existent cell viability test. True to the above stated fact, when the data sets using WST-8 assay were trained and tested for decision tree model to find out the attributes responsible for affecting the cytotoxicity in the cell cultures, it demonstrated comparable results. The hierarchical structure of the decision tree revealed identical attributes for first three splits, which were ZnO nanoparticles dose concentration, source of nanoparticles, and cell-line passage stage. The other attributes that featured in the decision tree were exposure time, size, and charge of the nanoparticles. Here again, the model illustrated an accuracy of 88.09%.

On cumulative analysis of the collective results attained in all the decision trees, ZnO dose concentration formed the root node maximum number of times, making it the most essential attribute responsible for causing cytotoxicity. This was followed by exposure time to ZnO nanoparticles. The next in line was the disease state of the cell lines,

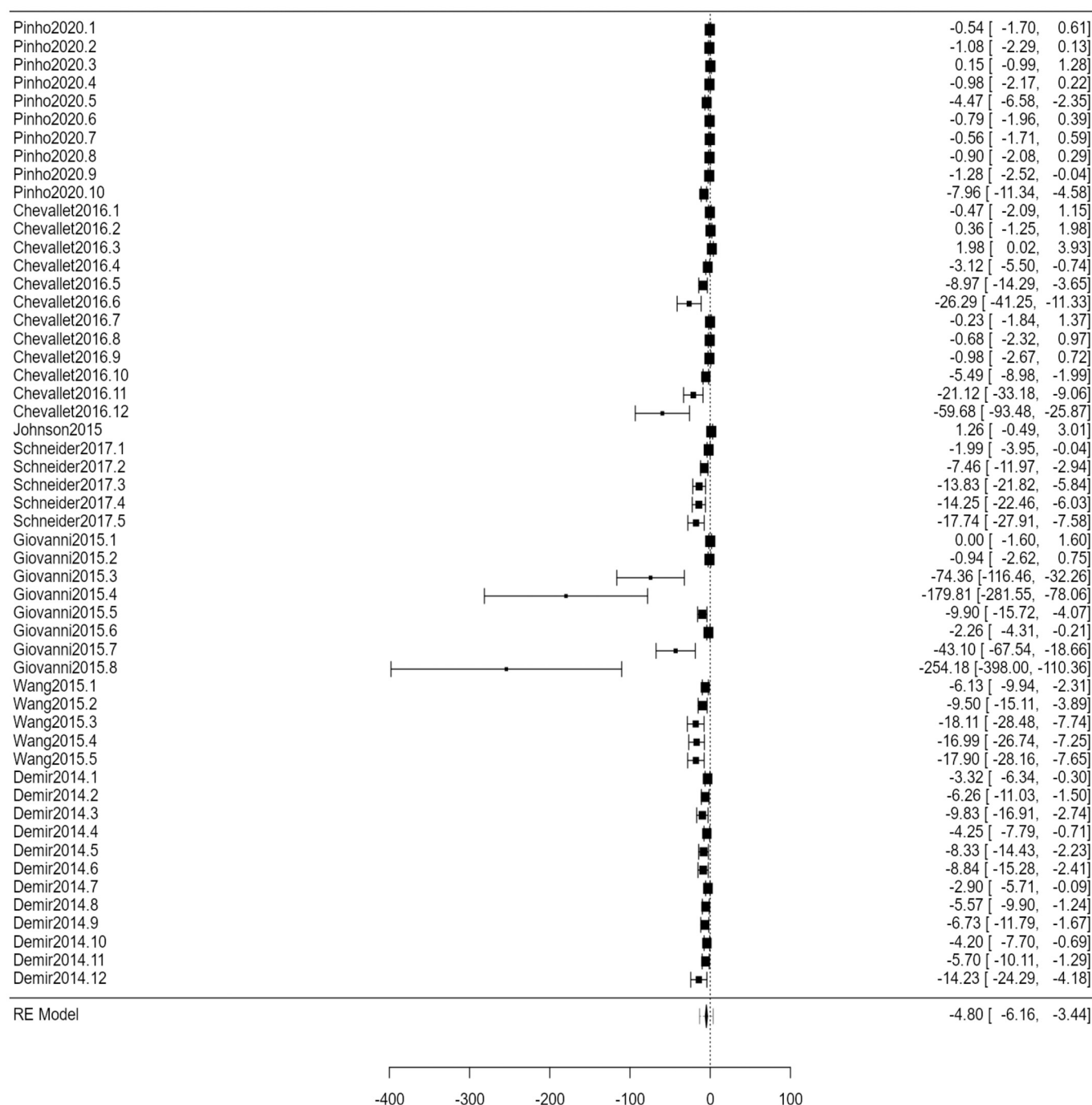


Fig. 8. Forest Plot Meta-analysis of the cytotoxic effects of ZnO nanoparticles vs. control using Trypan Blue Cell Viability Assay. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

where the cancerous cells were more prone to cytotoxicity as compared to the normal cells. Another attribute found quintessential, was the size of the nanoparticles used, followed by its charge. Rest of the attributes didn't show much effect, besides the source of nanoparticles and passage stage of the cells, as these attributes were a part of the leaf node, way down the hierarchy of the root node. All the models exhibited high accuracy rate despite training and test sets split into 7:3 ratio. The highest accuracy of 94.73% was found for Decision Tree made using Alamar Blue assay data points. The summarized results from the eight decision trees have been shown in Table 1.

Therefore, the order of the attributes showing profound effect on the

cytotoxicity profile of ZnO nanoparticles is:

ZnO nanoparticles' dose concentration > Exposure time > Disease state of the cell line
> Size of nanoparticles > charge of nanoparticles > source of nanoparticles >
Passage stage of cell-line

Upon closer examination of the vast scope of mined literature and different assumptions considered for every individual study, the conclusive statement regarding degree of influence of every possible attribute and their inter-linkages in determining cell viability when exposed to ZnO nanoparticles, was not reported till date. The forest plots with different data sets established cytotoxic nature of ZnO nanoparticles towards in vitro models unambiguously. Yet, the correlation

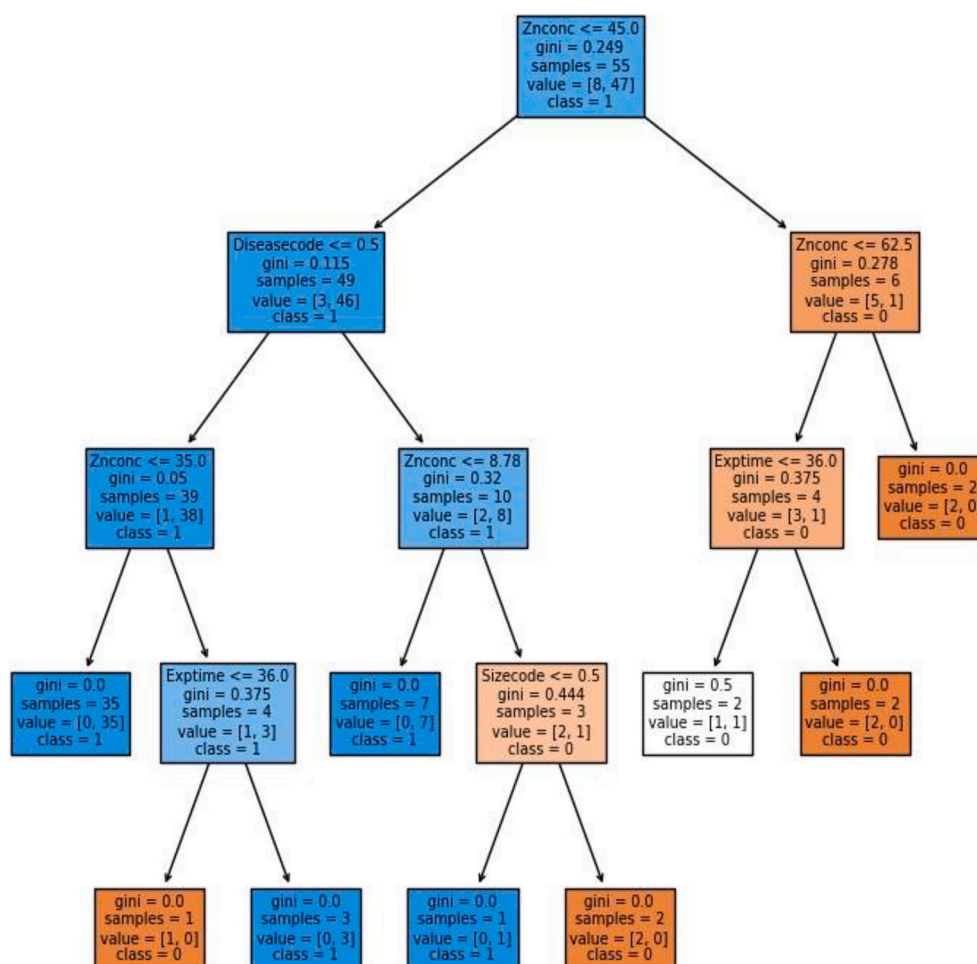


Fig. 9. Decision Tree for attributes responsible for causing cytotoxicity in data sets using Alamar Blue Cell Viability Assay, on exposure to ZnO nanoparticles. The model exhibited 94.73% accuracy rate, identifying 4 attributes responsible for causing cytotoxicity – nanoparticles' concentration, disease state of cells, exposure time and size of nanoparticles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

between different attributes and cytotoxicity posed by ZnO nanoparticles was only ascertained through decision trees.

It is well understood through literature that the cytotoxicity is all about dose of the nanoparticle and the exposure time. The multiple decision tree models created for varied data samples in the present study, agreed to the known truth. The elucidative review of articles reported that the number of nanoparticles, that penetrate the cells directly, depend on the molar concentration of nanoparticles in the adjacent medium multiplied by the exposure time, i.e., more the concentration, higher is the toxicity observed in the cell lines. This has also been validated by individual studies. For instance, Tobacco BY-2 cells exhibited hypersensitive signals, reduced cell growth, and enhanced oxidative stress when treated with increased concentration of ZnO nanoparticles (Balážová et al., 2020). In another study, A549 cells were subjected to nano-specific responses at three time points. Increased exposure resulted in higher cytotoxicity, leading to stress responses and cellular damage. The adverse responses heightened with increased concentration, but the intensity gradually reduced with prolonged exposure (Dekkers et al., 2018). Another important insight obtained through the results was to pair cytotoxicity posed by ZnO nanoparticles with the cell type(s) used for screening, as the viability results differed substantially with respect to those attributes. This has also been established by few individual studies showing varied cytotoxic effects of ZnO nanoparticles exposed to different cell lines, with constant physiochemical conditions (Li et al., 2012, 2020; Liang et al., 2018). Thus, decision trees can reveal critical variables from a complex dataset,

perform accurately as classifiers, and can be sufficiently employed for future use. In order to attain regulatory acceptance for our predictive model, the OECD guidelines were complied by, including, an explicit algorithm (decision tree), defined end point (predicting high versus low cell viability), a distinct domain of applicability (ZnO nanoparticles with same set of features), appropriate measure of the accuracy of the decision trees, and mechanistic discernment of the models.

3.6. Limitations

Our study addressed the need for developing a predictive model framework for potential cytotoxicity posed by ZnO nanoparticles. In spite of the comprehensive data compilation and extensive analysis to incorporate as many attributes as possible, there is still room for improvement. Primarily, there was publication bias identified in the Forest Plots. There are certain reasons which account for the biases, such as, the data was compiled from published articles related to toxicological analysis of ZnO nanoparticles, this categorically eliminated the inclusion of non-published data reporting no toxicity posed by these materials. Moreover, the articles highlighting applications of ZnO nanoparticles usually report no quantitative data for toxicity, as it is generally classified as non-significant. Therefore, the scope for attaining heterogeneous and diverse picture of research debating the cytotoxicity of ZnO nanoparticles remained unclear. Secondly, the data sets / experiments had missing values or lacked certain attributes which may be essential in comparing the significance of all the stated features. Some of

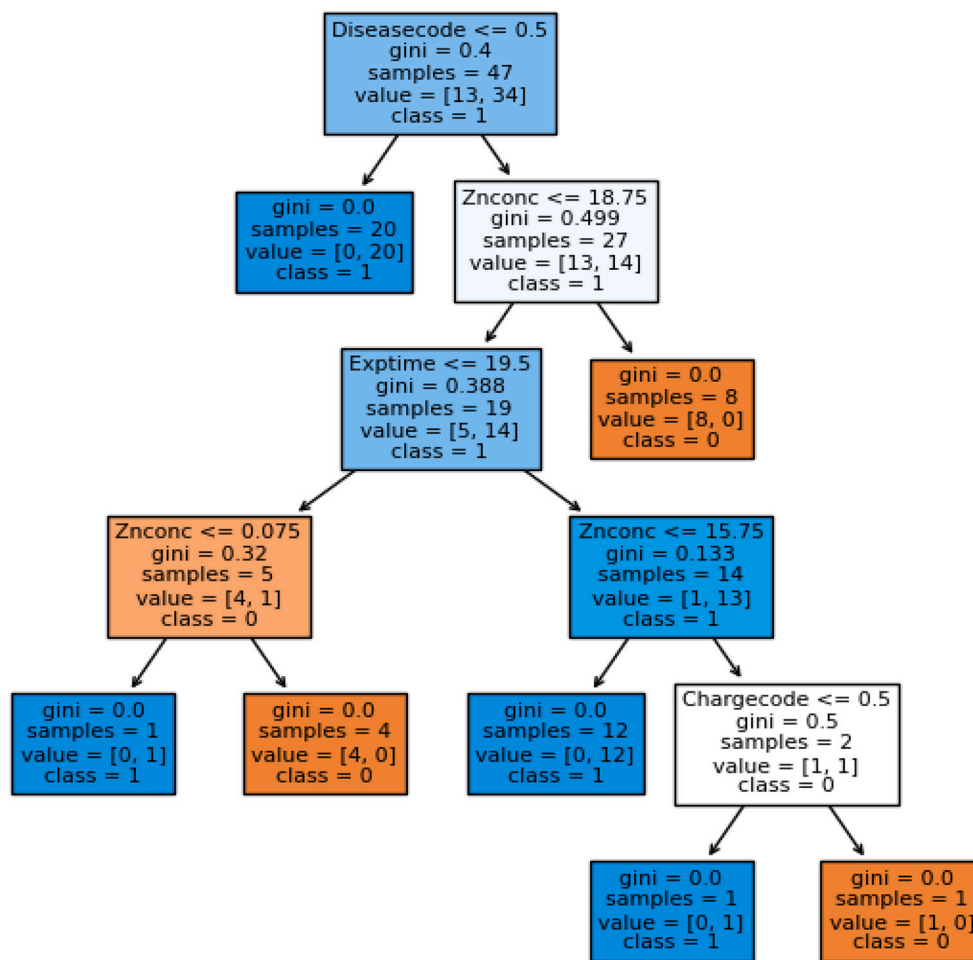


Fig. 10. Decision Tree defining attributes responsible for causing cytotoxicity on exposure to ZnO nanoparticles in the cell lines, using Trypan Blue Cell Viability Assay. The model exhibited 93.75% accuracy rate, identifying 4 attributes responsible for causing cytotoxicity, – disease state of the cells, concentration of nanoparticles, exposure time and charge on nanoparticles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Summarized results of attributes found responsible in causing cytotoxicity by ZnO nanoparticles in different decision trees.

Decision Tree	Cell Viability Assay	Model accuracy	Attributes influencing cell viability
Decision tree 1	Alamar Blue Assay	94.73%	ZnO dose conc., disease state of cells, exposure time, size of nanoparticles
Decision tree 2	MTT Assay	79.73%	ZnO dose conc., exposure time, disease state of the cells, size, charge and source of nanoparticles
Decision tree 3	NRU Assay	88.88%	ZnO dose conc., exposure time, size, charge, and source of nanoparticles, passage stage and species of origin of cell-line
Decision tree 4	WST-8 Assay	88.09%	ZnO dose conc., source of nanoparticles, passage stage of cell-line, exposure time, size and charge of nanoparticles
Decision tree 5	WST-1 Assay	81.08%	ZnO dose conc., source of nanoparticles, passage stage of cell-line, charge on nanoparticles, exposure time, size of nanoparticles
Decision tree 6	SRB Assay	82.23%	Exposure time, ZnO dose conc., source of nanoparticles, disease state of the cell-line
Decision tree 7	MTS Assay	75.98%	ZnO dose conc., exposure time, cell-line passage stage, charge, size, and source of nanoparticles
Decision tree 8	Trypan Blue Assay	93.75%	Disease state of the cells, ZnO dose conc., exposure time, charge on nanoparticles

these features that have been sparingly discussed in the previous studies include, aggregation effect of nanoparticles (Tripathy et al., 2014), aspect ratio and surface area effect (Yin and Casey, 2015), surface functionalization effect (Cao et al., 2020) and the effect of pre-exposure to nanoparticles (Vysloulzil et al., 2020). Most importantly, the cytotoxicity evaluation based on different cell viability assays cannot be compared at one pedestal. The results are not only influenced by the defined attributes, but there are other factors, such as intrinsic optical absorbance/scattering of light on exposure to varied dyes, enzymes and proteins employed in the cell viability assays. Thus, on clear analysis, though the research successfully concludes cytotoxic nature of ZnO nanoparticles to cell-lines, it is essential to reform the decision tree models for higher accuracy and more reliable predictions. Furthermore, the model may be improvised by performing cross validation of results through applying other machine learning algorithms.

4. Conclusion

Assessment of safety profile of any chemical/drug/nanoparticles begins with testing its impact on the most basic life form, that is, the cell. Identifying key properties contributing in causing cytotoxicity, estimating their degree of significance and correlation with other attributes forms the base for creating safety profile of any particle that may be exposed to humans, animals or the environment. >150 publications were mined to extract 1240 toxicity data points to perform a meta-analysis for evaluating cytotoxicity of ZnO nanoparticles in vitro. For each data point, 14 attributes were extracted, and the sample size for each data point was also identified. If we consider each sample as an individual experiment, then, the study contains a total of ($n = 4446$)

number of experiments, which validates the claim, being one of the most comprehensive datasets collected for ZnO nanoparticles till date. Forest plots and decision trees were used in order to perform the study concluding that ZnO nanoparticles' concentration, exposure time, disease state of the cell line and size of these nanoparticles are the most important attributes responsible for causing in vitro cytotoxicity (in descending order of their level of significance). This establishes beyond doubt that one of the most comprehensively used nanoparticle, which has gained acceptance in variety of day-to-day products, needs extensive testing and prediction framework for its available set of physiochemical features, before the manufacturer uses it in the industrial scale production. The toxicity screening has shown detrimental effects in number of in vivo models as well humans with a large body of evidence waiting to be explored. Though, there are few studies analyzing the direct effect of these nanoparticles on human volunteers, the researchers had failed to reach a common consensus. Where sunscreen containing nano-sized ZnO did not exhibit local toxicity, workers working in the zinc production and processing of galvanized sheet steel exposed to a complex mixture of particles and gases along with ZnO nanoparticles, revealed airway inflammation on inhalation (Mohammed et al., 2019; Monsé et al., 2019). But the scope of the study does not end here. The cytotoxicity has been explored but there is long way to go for genotoxicity, neurotoxicity and other types of toxicity which may be caused by ZnO nanoparticles. In addition to this, the model can be extended to incorporate in vivo studies with different model organisms.

At this point in time, the world is in a very pivotal phase, and there are more concerns than ever about the safety of products. Based upon toxicological studies and long-term effects of these nanoparticles, the decisions made today will have a long-lasting impact on our future generations. Therefore, it's need of the hour to critically check and rigorously analyze for any adverse consequence of products that are introduced in the market. So, for better environment and healthy individuals, toxicology research ought to be weighed and regarded at the higher pedestal than the applications itself.

Declaration of Competing Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript. The authors declare that they have no conflict of interest.

Acknowledgement

The authors acknowledge DBT Builder Programme (BT/INF/22/SP41295/2020) at Panjab University for financial assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tiv.2022.105418>.

References

Abbasi-Oshaghi, E., Mirzaei, F., Mirzaei, A., 2018. Effects of ZnO nanoparticles on intestinal function and structure in normal/high fat diet-fed rats and Caco-2 cells. *Nanomedicine (London)* 13. <https://doi.org/10.2217/NNM-2018-0202>.

Afantitis, A., Melagraki, G., Isigoni, P., Tsoumanis, A., Danai, D., Valsami-jones, E., Papadiamantis, A., Ellis, L.A., Sarimveis, H., Doganis, P., Karatzas, P., Tsiros, P., Liampa, I., Lobaskin, V., Greco, D., Serra, A., Anelli, P., Kinares, S., Aliisa, L., Grafström, R., Kohonen, P., Nymark, P., Willighagen, E., Puzyn, T., Rybinska-fryca, A., Lyubartsev, A., Alstrup, K., Gerit, J., Lofts, S., Svendsen, C., Harrison, S., Maier, D., Tamm, K., Jänes, J., Sikk, L., Dusinska, M., Longhin, E., Rundén-pran, E., Mariussen, E., El, N., Unger, W., Radnik, J., Tropsha, A., Cohen, Y., Leszczynski, J., Ogilvie, C., Wiesner, M., Winkler, D., Suzuki, N., Hyun, T., Choi, J., Sanabria, N., Gulumian, M., Lynch, I., 2020. NanoSolveIT project : driving nanoinformatics research to develop innovative and integrated tools for in silico nanosafety assessment. *Comput. Struct. Biotechnol. J.* 18, 583–602. <https://doi.org/10.1016/j.csbj.2020.02.023>.

Ahmad, J., Wahab, R., Siddiqui, M.A., Saquib, Q., Al-Khedhairi, A.A., 2020. Cytotoxicity and cell death induced by engineered nanostructures (quantum dots and nanoparticles) in human cell lines. *JBIC J. Biol. Inorg. Chem.* 325–338. <https://doi.org/10.1007/S00775-020-01764-5>, 2020 252 25.

Al-Ali, A.A.A., Al-Tamimi, S.Q., Al-Maliki, S.J., Abdullah, M.A., 2022. Toxic effects of zinc oxide nanoparticles and histopathological and caspase-9 expression changes in the liver and lung tissues of male mice model. *Appl. Nanosci.* 2021 122 12, 193–203. <https://doi.org/10.1007/S13204-021-02248-X>.

Alarifi, S., Ali, D., Alkahtani, S., Verma, A., Ahamed, M., Ahmed, M., Alhadlaq, H., 2013. Induction of oxidative stress, DNA damage, and apoptosis in a malignant human skin melanoma cell line after exposure to zinc oxide nanoparticles. *Int. J. Nanomedicine* 8, 983–993. <https://doi.org/10.2147/IJN.S42028>.

Almutairi, B., Albahser, G., Almeer, R., Alyami, N.M., Almutkhafi, H., Yaseen, K.N., Alkahtani, S., Alarifi, S., Saxena, M., 2020. Investigation of cytotoxicity apoptotic and inflammatory responses of biosynthesized zinc oxide nanoparticles from ocimum sanctum linn in human skin keratinocyte (Hacat) and human lung epithelial (A549) cells. *Oxidative Med. Cell. Longev.* 2020 <https://doi.org/10.1155/2020/1835475>.

Ankit Rohatgi, n.d. WebPlotDigitizer.

Attarilar, S., Yang, Jinfan, Ebrahimi, M., Wang, Q., Liu, J., Tang, Y., Yang, Junlin, 2020. The toxicity phenomenon and the related occurrence in metal and metal oxide nanoparticles: a brief review from the biomedical perspective. *Front. Bioeng. Biotechnol.* 8 <https://doi.org/10.3389/fbioe.2020.00822>.

Baek, M., Kim, M.K., Cho, H.J., Lee, J.A., Yu, J., Chung, H.E., Choi, S.J., 2011. Factors influencing the cytotoxicity of zinc oxide nanoparticles: particle size and surface charge. *J. Phys. Conf. Ser.* 304, 012044 <https://doi.org/10.1088/1742-6596/304/1/012044>.

Bai, K., Chuang, K., Ma, C., Chang, T., Chuang, H., 2017. Human lung adenocarcinoma cells with an EGFR mutation are sensitive to non-autophagic cell death induced by zinc oxide and aluminium-doped zinc oxide nanoparticles. *J. Toxicol. Sci.* 42, 437–444. <https://doi.org/10.2131/JTS.42.437>.

Balázová, L., Baláz, M., Babula, P., 2020. Zinc oxide nanoparticles damage tobacco BY-2 cells by oxidative stress followed by processes of autophagy and programmed cell death. *Nanomaterials* 10, 1066. <https://doi.org/10.3390/nano10061066>.

Bandeira, M., Possan, A.L., Pavin, S.S., Raota, C.S., Vebber, M.C., Giovanela, M., Roesch-Ely, M., Devine, D.M., Crespo, S., 2020. Mechanism of formation, characterization and cytotoxicity of green synthesized zinc oxide nanoparticles obtained from Ilex paraguariensis leaves extract. *Nano-Struct. Nano-Objects.* 24 <https://doi.org/10.1016/J.NANOSO.2020.100532>.

Cao, Y., Roursgaard, M., Keramanizadeh, A., Loft, S., Møller, P., 2015. Synergistic effects of zinc oxide nanoparticles and fatty acids on toxicity to caco-2 cells. *Int. J. Toxicol.* 34, 67–76. <https://doi.org/10.1177/1091581814560032>.

Cao, D., Shu, X., Zhu, D., Liang, S., Hasan, M., Gong, S., 2020. Lipid-coated ZnO nanoparticles synthesis, characterization and cytotoxicity studies in cancer cell. *Nano Conver.* 7, 1–18. <https://doi.org/10.1186/s40580-020-00224-9>.

Carmo, I.A.D., de Souza, A.K.N., Fayer, L., Munk, M., de Brandão, H.M., de Oliveira, L.F.C., Bandeira, S., Cavallini, G.S., de Souza, N.L.G.D., 2022. Cytotoxicity and Bactericidal Activity of Alginate/Polyethyleneglycol Films with Zinc Oxide or Silicon Oxide Nanoparticles for Food Packaging, pp. 1–12. <https://doi.org/10.1080/00914037.2022.2032706>.

Chauhan, R., Kumar, Amit, Tripathi, R., Kumar, Akhilesh, 2021. Advancing of zinc oxide nanoparticles for cosmetic applications. *Handb. Consum. Nanoprod.* 1–16 https://doi.org/10.1007/978-981-15-6453-6_100-1.

Chen, J., Ho, C., Chang, H., Lin, J., Yang, C., Tsai, M., Tsai, H., Lin, P., 2015. Particulate nature of inhaled zinc oxide nanoparticles determines systemic effects and mechanisms of pulmonary inflammation in mice. *Nanotoxicology* 9, 43–53. <https://doi.org/10.3109/17435390.2014.886740>.

Chen, P., Wang, H., He, M., Chen, B., Yang, B., Hu, B., 2019. Size-dependent cytotoxicity study of ZnO nanoparticles in HepG2 cells. *Ecotoxicol. Environ. Saf.* 171, 337–346. <https://doi.org/10.1016/j.ecoenv.2018.12.096>.

Chen, Y.Y., Lee, Y.H., Wang, B.J., Chen, R.J., Wang, Y.J., 2022. Skin damage induced by zinc oxide nanoparticles combined with UVB is mediated by activating cell pyroptosis via the NLRP3 inflammasome-autophagy-exosomal pathway. *Part. Fibre Toxicol.* 19, 1–22. <https://doi.org/10.1186/S12989-021-00443-W/FIGURES/9>.

Chevallet, M., Gallet, B., Fuchs, A., Jouneau, P., Um, K., Mintz, E., Michaud-Soret, I., 2016. Metal homeostasis disruption and mitochondrial dysfunction in hepatocytes exposed to sub-toxic doses of zinc oxide nanoparticles. *Nanoscale* 8, 18495–18506. <https://doi.org/10.1039/C6NR05306H>.

Czyżowska, A., Barbasz, A., 2020. A Review: Zinc Oxide Nanoparticles – Friends or Enemies? <https://doi.org/10.1080/09603123.2020.1805415>.

Danielsen, P., Cao, Y., Roursgaard, M., Møller, P., Loft, S., 2015. Endothelial cell activation, oxidative stress and inflammation induced by a panel of metal-based nanomaterials. *Nanotoxicology* 9, 813–824. <https://doi.org/10.3109/17435390.2014.980449>.

De Angelis, I., Barone, F., Zijno, A., Bizzarri, L., Russo, M.T., Pozzi, R., Franchini, F., Giudetti, G., Uboldi, C., Ponti, J., Rossi, F., De Berardis, B., 2013. Comparative study of ZnO and TiO2 nanoparticles: physicochemical characterisation and toxicological effects on human colon carcinoma cells. *Nanotoxicology* 7, 1361–1372. <https://doi.org/10.3109/17435390.2012.741724>.

Dekkers, S., Williams, T.D., Zhang, J., Zhou, J., Vandebriel, R.J., De La Fonteyne, L.J.J., Gremmer, E.R., He, S., Guggenheim, E.J., Lynch, I., Cassee, F.R., De Jong, W.H., Viant, M.R., 2018. Multi-omics approaches confirm metal ions mediate the main toxicological pathways of metal-bearing nanoparticles in lung epithelial A549 cells. *Environ. Sci. Nano* 5, 1506–1517. <https://doi.org/10.1039/c8en00071a>.

Demir, E., Akça, H., Kaya, B., Burgucu, D., Tokgün, O., Turna, F., Aksakal, S., Vales, G., Creus, A., Marcos, R., 2014. Zinc oxide nanoparticles: genotoxicity, interactions with

- UV-light and cell-transforming potential. *J. Hazard. Mater.* 264, 420–429. <https://doi.org/10.1016/J.JHAZMAT.2013.11.043>.
- El-Ansary, M.S.M., Hamouda, R.A., Elshamy, M.M., 2021. Using biosynthesized zinc oxide nanoparticles as a pesticide to alleviate the toxicity on Banana infested with parasitic-nematode. *Waste Biomass Valoriz.* 2021 131 13, 405–415. <https://doi.org/10.1007/S12649-021-01527-6>.
- Fouda, A., EL-Din Hassan, S., Salem, S.S., Shaheen, T.I., 2018. In-vitro cytotoxicity, antibacterial, and UV protection properties of the biosynthesized zinc oxide nanoparticles for medical textile applications. *Micro. Pathog.* 125, 252–261. <https://doi.org/10.1016/J.MICPATH.2018.09.030>.
- Fu, J., Zeng, X., He, N., 2017. Comparative cytotoxicity induced by zinc oxide nanoparticles in human prostate cells. *J. Nanosci. Nanotechnol.* 17, 196–202. <https://doi.org/10.1166/JNN.2017.12444>.
- Fukui, H., Horie, H., Endoh, S., Kato, H., Fujita, K., Nishio, K., Komaba, L., Maru, J., Miyauhi, A., Nakamura, A., Kinugasa, S., Yoshida, Y., Hagiwara, Y., Iwahashi, H., 2012. Association of zinc ion release and oxidative stress induced by intratracheal instillation of ZnO nanoparticles to rat lung. *Chem. Biol. Interact.* 198, 29–37. <https://doi.org/10.1016/J.CBI.2012.04.007>.
- Furxhi, I., Murphy, F., Mullins, M., Arvanitis, A., Poland, C.A., 2020. Nanotoxicology data for in silico tools: a literature review. *Nanotoxicology* 14, 612–637. <https://doi.org/10.1080/17435390.2020.1729439>.
- Gao, F., Ma, N., Zhou, H., Wang, Q., Zhang, H., Wang, P., Hou, H., Wen, H., Li, L., 2016. Zinc oxide nanoparticles-induced epigenetic change and G2/M arrest are associated with apoptosis in human epidermal keratinocytes. *Int. J. Nanomedicine* 11, 3859. <https://doi.org/10.2147/IJN.S107021>.
- Gernand, J.M., Casman, E.A., 2014. A meta-analysis of carbon nanotube pulmonary toxicity studies-how physical dimensions and impurities affect the toxicity of carbon nanotubes. *Risk Anal.* 34, 583–597. <https://doi.org/10.1111/risa.12109>.
- Giovanni, M., Tay, C., Setyawati, M., Xie, J., Ong, C., Fan, R., Yue, J., Zhang, L., Leong, D., 2015. Toxicity profiling of water contextual zinc oxide, silver, and titanium dioxide nanoparticles in human oral and gastrointestinal cell systems. *Environ. Toxicol.* 30, 1459–1469. <https://doi.org/10.1002/TOX.22015>.
- Global Nanotechnology Industry [WWW Document], n.d. URL <https://www.globenewswire.com/news-release/2020/07/16/2062964/0/en/Global-Nanotechnology-Industry.html> (accessed 4.25.21).
- Gong, Y., Ji, Y., Liu, F., Li, J., Cao, Y., 2017. Cytotoxicity, oxidative stress and inflammation induced by ZnO nanoparticles in endothelial cells: interaction with palmitate or lipopolysaccharide. *J. Appl. Toxicol.* 37, 895–901. <https://doi.org/10.1002/JAT.3415>.
- Gopala Krishna, P., Ananthaswamy, P., Trivedi, P., Chaturvedi, V., Bhangi Mutta, N., Sannaiah, A., Erra, A., Yadavalli, T., 2017. Antitubercular activity of ZnO nanoparticles prepared by solution combustion synthesis using lemon juice as bio-fuel. *Mater. Sci. Eng. C Mater. Biol. Appl.* 75, 1026–1033. <https://doi.org/10.1016/J.MSEC.2017.02.093>.
- Guo, D., Bi, H., Wu, Q., Wang, D., Cui, Y., 2013. Zinc oxide nanoparticles induce rat retinal ganglion cell damage through bcl-2, caspase-9 and caspase-12 pathways. *J. Nanosci. Nanotechnol.* 13, 3769–3777. <https://doi.org/10.1166/JNN.2013.7169>.
- Han, Z., Yan, Q., Ge, W., Liu, Z., Gurunathan, S., De Felici, M., Shen, W., Zhang, X., 2016. Cytotoxic effects of ZnO nanoparticles on mouse testicular cells. *Int. J. Nanomedicine* 11, 5187–5203. <https://doi.org/10.2147/IJN.S111447>.
- He, T., Long, J., Li, J., Liu, L., Cao, Y., 2017. Toxicity of ZnO nanoparticles (NPs) to A549 cells and A549 epithelium in vitro: interactions with dipalmitoyl phosphatidylcholine (DPPC). *Environ. Toxicol. Pharmacol.* 56, 233–240. <https://doi.org/10.1016/J.ETAP.2017.10.002>.
- Heng, B., Zhao, X., Xiong, S., Ng, K., Boey, F., Loo, J., 2010. Toxicity of zinc oxide (ZnO) nanoparticles on human bronchial epithelial cells (BEAS-2B) is accentuated by oxidative stress. *Food Chem. Toxicol.* 48, 1762–1766. <https://doi.org/10.1016/J.FCT.2010.04.023>.
- Ho, C., Lee, H., Chen, C., Luo, Y., Tsai, M., Tsai, H., Lin, P., 2017. Involvement of the cytokine-IDO1-AhR loop in zinc oxide nanoparticle-induced acute pulmonary inflammation. *Nanotoxicology* 11, 360–370. <https://doi.org/10.1080/17435390.2017.1306129>.
- Holmes, A.M., Kempson, I., Turnbull, T., Paterson, D., Roberts, M.S., 2020a. Penetration of zinc into human skin after topical application of nano zinc oxide used in commercial sunscreen formulations. *ACS Appl. Bio. Mater.* 3, 3640–3647. <https://doi.org/10.1021/ACSABM.0C00280>.
- Holmes, A., Mackenzie, L., Roberts, M., 2020b. Disposition and measured toxicity of zinc oxide nanoparticles and zinc ions against keratinocytes in cell culture and viable human epidermis. *Nanotoxicology* 14, 263–274. <https://doi.org/10.1080/17435390.2019.1692382>.
- Husain, F.M., Qais, F.A., Ahmad, I., Hakeem, M.J., Baig, M.H., Khan, J.M., Al-Shabib, N.A., 2022. Biosynthesized zinc oxide nanoparticles disrupt established biofilms of pathogenic bacteria. *Appl. Sci.* 2022 12. <https://doi.org/10.3390/APP12020710> page 710 12, 710.
- Ickrath, P., Wagner, M., Scherzad, A., Gehrke, T., Burghartz, M., Hagen, R., Radeloff, K., Kleinsasser, N., Hackenberg, S., 2017. Time-dependent toxic and genotoxic effects of zinc oxide nanoparticles after Long-term and repetitive exposure to human mesenchymal stem cells. *Int. J. Environ. Res. Public Health* 14. <https://doi.org/10.3390/IJERPH14121590>.
- Jain, A.K., Singh, D., Dubey, K., Maurya, R., Pandey, A.K., 2019. Zinc oxide nanoparticles induced gene mutation at the GPR1 locus and cell cycle arrest associated with apoptosis in V-79 cells. *J. Appl. Toxicol.* 39, 735–750. <https://doi.org/10.1002/JAT.3763>.
- Jeong, S., Kim, H., Ryu, H., Ryu, W., Park, Y., Bae, H., Jang, Y., Son, S., 2013. ZnO nanoparticles induce TNF- α expression via ROS-ERK-Egr-1 pathway in human keratinocytes. *J. Dermatol. Sci.* 72, 263–273. <https://doi.org/10.1016/J.JDERMSCI.2013.08.002>.
- Joachimaki, M.P., 2021. Zinc against COVID-19? Symptom surveillance and deficiency risk groups. *PLoS Negl. Trop. Dis.* 15, e0008895. <https://doi.org/10.1371/JOURNAL.PNTD.0008895>.
- Johnson, B., Fraietta, J., Gracias, D., Hope, J., Stairiker, C., Patel, P., Mueller, Y., McHugh, M., Jablonowski, L., Wheatley, M., Katsikis, P., 2015. Acute exposure to ZnO nanoparticles induces autophagic immune cell death. *Nanotoxicology* 9, 737–748. <https://doi.org/10.3109/17435390.2014.974709>.
- Kang, T., Guan, R., Chen, X., Song, Y., Jiang, H., Zhao, J., 2013. In vitro toxicity of different-sized ZnO nanoparticles in Caco-2 cells. *Nanoscale Res. Lett.* 8, 1–8. <https://doi.org/10.1186/1556-276X-8-496>.
- Kaur, J., Sood, K., Bhardwaj, N., Arya, S.K., Khatri, M., 2020. Nanomaterial loaded chitosan nanocomposite films for antimicrobial food packaging. In: *Materials Today: Proceedings*. Elsevier Ltd, pp. 1904–1909. <https://doi.org/10.1016/j.matpr.2020.05.309>.
- Khan, M.A., Yasmin, H., Shah, Z.A., Rinklebe, J., Alyemeni, M.N., Ahmad, P., 2022. Co application of biofertilizer and zinc oxide nanoparticles upregulate protective mechanism culminating improved arsenic resistance in maize. *Chemosphere* 294, 133796. <https://doi.org/10.1016/J.CHEMOSPHERE.2022.133796>.
- Kim, A., Ahmed, F., Jung, G., Cho, S., Kim, D., Um, S., 2013. Hepatocyte cytotoxicity evaluation with zinc oxide nanoparticles. *J. Biomed. Nanotechnol.* 9, 926–929. <https://doi.org/10.1166/JBN.2013.1495>.
- Kim, C., Nguyen, H., Ignacio, R., Kim, J., Cho, H., Maeng, E., Kim, Y., Kim, M., Park, B., Kim, S., 2014. Immunotoxicity of zinc oxide nanoparticles with different size and electrostatic charge. *Int. J. Nanomedicine* 9 (Suppl. 2), 195–205. <https://doi.org/10.2147/IJN.S57935>.
- Kononenko, V., Repar, N., Marušić, N., Drašler, B., Romih, T., Hočevar, S., Drobne, D., 2017. Comparative in vitro genotoxicity study of ZnO nanoparticles, ZnO macroparticles and ZnCl₂ to MDCK kidney cells: size matters. *Toxicol. in Vitro* 40, 256–263. <https://doi.org/10.1016/J.TIV.2017.01.015>.
- Król, A., Pomastowski, P., Rafińska, K., Railean-Plugaru, V., Buszewski, B., 2017. Zinc oxide nanoparticles: synthesis, antiseptic activity and toxicity mechanism. *Adv. Colloid Interf. Sci.* 249, 37–52. <https://doi.org/10.1016/J.CIS.2017.07.033>.
- Labouta, H.I., Asgarian, N., Rinker, K., Cramb, D.T., 2019. Meta-analysis of nanoparticle cytotoxicity via data-mining the literature. *ACS Nano* 13, 1583–1594. <https://doi.org/10.1021/acsnano.8b07562>.
- Lai, X., Wei, Y., Zhao, H., Chen, S., Bu, X., Lu, F., Qu, D., Yao, L., Zheng, J., Zhang, J., 2015. The effect of Fe₂O₃ and ZnO nanoparticles on cytotoxicity and glucose metabolism in lung epithelial cells. *J. Appl. Toxicol.* 35, 651–664. <https://doi.org/10.1002/JAT.3128>.
- Lee, P., Chen, B., Gollavelli, G., Shen, S., Yin, Y., Lei, S., Jhang, C., Lee, W., Ling, Y., 2014. Development and validation of TOF-SIMS and CLSM imaging method for cytotoxicity study of ZnO nanoparticles in HaCat cells. *J. Hazard. Mater.* 277, 3–12. <https://doi.org/10.1016/J.JHAZMAT.2014.03.046>.
- Li, C., Shen, C., Cheng, Y., Huang, S., Wu, C., Kao, C., Liao, J., Kang, J., 2012. Organ biodistribution, clearance, and genotoxicity of orally administered zinc oxide nanoparticles in mice. *Nanotoxicology* 6, 746–756. <https://doi.org/10.3109/17435390.2011.620717>.
- Li, J., Song, Y., Vogt, R., Liu, Y., Luo, J., Li, T., 2020. Bioavailability and cytotoxicity of cerium- (IV), copper- (II), and zinc oxide nanoparticles to human intestinal and liver cells through food. *Sci. Total Environ.* 702. <https://doi.org/10.1016/J.SCTOTENV.2019.134700>.
- Liam Critchley, 2020. Metal Oxide Nanoparticles – Are they Safe? [WWW Document]. URL: <https://www.azonano.com/article.aspx?ArticleID=5444> (accessed 5.11.21).
- Liang, H., Chen, A., Lai, X., Liu, J., Wu, J., Kang, Y., Wang, X., Shao, L., 2018. Neuroinflammation is induced by tongue-instilled ZnO nanoparticles via the Ca²⁺-dependent NF- κ B and MAPK pathways. *Part. Fibre Toxicol.* 15. <https://doi.org/10.1186/S12989-018-0274-0>.
- Ling, C., An, H., Li, L., Wang, J., Lu, T., Wang, H., Hu, Y., Song, G., Liu, S., 2021. Genotoxicity evaluation of titanium dioxide nanoparticles in vitro: a systematic review of the literature and Meta-analysis. *Biol. Trace Elem. Res.* 199, 2057–2076. <https://doi.org/10.1007/s12011-020-02311-8>.
- Liu, Q., Xu, C., Ji, G., Liu, H., Mo, Y., Tollerud, D., Gu, A., Zhang, Q., 2016. Sublethal effects of zinc oxide nanoparticles on male reproductive cells. *Toxicol. in Vitro* 35, 131–138. <https://doi.org/10.1016/J.TIV.2016.05.017>.
- Liu, Z., Lv, X., Xu, L., Liu, X., Zhu, X., Song, E., Song, Y., 2020. Zinc oxide nanoparticles effectively regulate autophagic cell death by activating autophagosome formation and interfering with their maturation. *Part. Fibre Toxicol.* 1–17. <https://doi.org/10.1186/S12989-020-00379-7>, 2020 171 17.
- Mantecca, P., Kasemets, K., Deokar, A., Perelshtein, I., Gedanken, A., Bahk, Y.K., Kianfar, B., Wang, J., 2017. Airborne nanoparticle release and toxicological risk from metal-oxide-coated textiles: toward a multiscale safe-by-design approach. *Environ. Sci. Technol.* 51, 9305–9317. <https://doi.org/10.1021/ACS.EST.7B02390>.
- Medici, S., Peana, M., Pelucelli, A., Zoroddu, M.A., 2021. An updated overview on metal nanoparticles toxicity. *Semin. Cancer Biol.* 76, 17–26. <https://doi.org/10.1016/J.SEMCANCER.2021.06.020>.
- Miri, A., Khatami, M., Ebrahimi, O., Sarani, M., . Cytotoxic and Antifungal Studies of Biosynthesized Zinc Oxide Nanoparticles using Extract of *Prosopis farcta* Fruit. <http://mc.manuscriptcentral.com/tgcl>, 13, pp. 27–33. <https://doi.org/10.1080/17518253.2020.1717005>.
- Mohammed, Y.H., Holmes, A., Haridass, I.N., Sanchez, W.Y., Studier, H., Grice, J.E., Benson, H.A.E., Roberts, M.S., 2019. Support for the safe use of zinc oxide nanoparticle sunscreens: lack of skin penetration or cellular toxicity after repeated application in volunteers. *J. Invest. Dermatol.* 139, 308–315. <https://doi.org/10.1016/j.jid.2018.08.024>.

- Monsé, C., Raulf, M., Hagemeyer, O., Van Kampen, V., Kendzia, B., Gering, V., Marek, E. M., Jettkant, B., Bünger, J., Merget, R., Brüning, T., 2019. Airway inflammation after inhalation of nano-sized zinc oxide particles in human volunteers. *BMC Pulm. Med.* 19, 1–11. <https://doi.org/10.1186/s12890-019-1026-0>.
- Moratin, H., Scherzad, A., Gehrke, T., Ickrath, P., Radeloff, K., Kleinsasser, N., Hackenberg, S., 2018. Toxicological characterization of ZnO nanoparticles in malignant and non-malignant cells. *Environ. Mol. Mutagen.* 59, 247–259. <https://doi.org/10.1002/EM.22156>.
- Namvar, F., Rahman, H.S., Mohamad, R., Azizi, S., Tahir, P.M., Chartrand, M.S., Yeap, S. K., 2015. Cytotoxic effects of biosynthesized zinc oxide nanoparticles on murine cell lines. Evidence-based Complement. Altern. Med. 2015 <https://doi.org/10.1155/2015/593014>.
- Ng, C., Yong, L., Hande, M., Ong, C., Yu, L., Bay, B., Baeg, G., 2017. Zinc oxide nanoparticles exhibit cytotoxicity and genotoxicity through oxidative stress responses in human lung fibroblasts and *Drosophila melanogaster*. *Int. J. Nanomedicine* 12, 1621–1637. <https://doi.org/10.2147/IJN.S124403>.
- Oh, E., Liu, R., Nel, A., Gemill, K.B., Bilal, M., Cohen, Y., Medintz, I.L., 2016. Meta-analysis of cellular toxicity for cadmium-containing quantum dots. *Nat. Nanotechnol.* 11, 479–486. <https://doi.org/10.1038/nnano.2015.338>.
- Pal, A., Squitti, R., Picozza, M., Pawar, A., Rongioletti, M., Dutta, A.K., Sahoo, S., Goswami, K., Sharma, P., Prasad, R., 2020. Zinc and COVID-19: basis of current clinical trials. *Biol. Trace Elem. Res.* 2882–2892. <https://doi.org/10.1007/S12011-020-02437-9>, 2020 1998 199.
- Pati, R., Das, I., Mehta, R., Sahu, R., Sonawane, A., 2016. Zinc-oxide nanoparticles exhibit genotoxic, clastogenic, cytotoxic and actin depolymerization effects by inducing oxidative stress responses in macrophages and adult mice. *Toxicol. Sci.* 150, 454–472. <https://doi.org/10.1093/TOXSCI/KFW010>.
- Pinho, A., Martins, F., Costa, M., Senos, A., Silva, O., Pereira, M., Rebelo, S., 2020. In vitro cytotoxicity effects of zinc oxide nanoparticles on spermatogonia cells. *Cells* 9. <https://doi.org/10.3390/CELLS9051081>.
- Poier, N., Hochstötger, J., Hackenberg, S., Scherzad, A., Bregenzer, M., Schopper, D., Kleinsasser, N., 2020. Effects of zinc oxide nanoparticles in HUVEC: cyto- and genotoxicity and functional impairment after long- term and repetitive exposure in vitro. *Int. J. Nanomedicine* 15, 4441. <https://doi.org/10.2147/IJN.S246797>.
- Prajitha, N., Athira, S.S., Mohanan, P.V., 2019. Bio-interactions and risks of engineered nanoparticles. *Environ. Res.* <https://doi.org/10.1016/j.envres.2019.02.003>.
- Qin, X., Tang, Q., Jiang, X., Zhang, J., Wang, B., Liu, X., Zhang, Y., Zou, Z., Chen, C., 2020. Zinc oxide nanoparticles induce Ferroptotic neuronal cell death in vitro and in vivo. *Int. J. Nanomedicine* 15, 5299–5315. <https://doi.org/10.2147/IJN.S250367>.
- Safar, R., Doumandji, Z., Saidou, T., Ferrari, L., Nahle, S., Rihn, B., Joubert, O., 2019. Cytotoxicity and global transcriptional responses induced by zinc oxide nanoparticles NM 110 in PMA-differentiated THP-1 cells. *Toxicol. Lett.* 308, 65–73. <https://doi.org/10.1016/j.TOXLET.2018.11.003>.
- Sahu, D., Kannan, G.M., Vijayaraghavan, R., 2014. Size-Dependent Effect of Zinc Oxide on Toxicity and Inflammatory Potential of Human Monocytes, 77, pp. 177–191. <https://doi.org/10.1080/15287394.2013.853224>.
- Saptarshi, S., Feltis, B., Wright, P., Lopata, A., 2015. Investigating the immunomodulatory nature of zinc oxide nanoparticles at sub-cytotoxic levels in vitro and after intranasal instillation in vivo. *J. Nanobiotechnol.* 13 <https://doi.org/10.1186/S12951-015-0067-7>.
- Schneider, T., Westermann, M., Gleit, M., 2017. In vitro uptake and toxicity studies of metal nanoparticles and metal oxide nanoparticles in human HT29 cells. *Arch. Toxicol.* 91, 3517–3527. <https://doi.org/10.1007/S00204-017-1976-Z>.
- Shalini, D., Senthilkumar, S., Rajaguru, P., 2018. Effect of size and shape on toxicity of zinc oxide (ZnO) nanomaterials in human peripheral blood lymphocytes. *Toxicol. Mech. Methods* 28, 87–94. <https://doi.org/10.1080/15376516.2017.1366609>.
- Sharma, V., Anderson, D., Dhawan, A., 2012. Zinc oxide nanoparticles induce oxidative DNA damage and ROS- triggered mitochondria mediated apoptosis in human liver cells (HepG2). *Apoptosis* 17, 852–870. <https://doi.org/10.1007/S10495-012-0705-6>.
- Sharma, A., Singh, V., Gera, R., Purohit, M., Ghosh, D., 2017. Zinc oxide nanoparticle induces microglial death by NADPH-oxidase-independent reactive oxygen species as well as energy depletion. *Mol. Neurobiol.* 54, 6273–6286. <https://doi.org/10.1007/S12035-016-0133-7>.
- Sharma, A., Gorey, B., Casey, A., 2018. In Vitro Comparative Cytotoxicity Study of Aminated Polystyrene, Zinc Oxide and Silver Nanoparticles on a Cervical Cancer Cell Line, 42, pp. 9–23. <https://doi.org/10.1080/01480545.2018.1424181>.
- Sharma, A., Sood, K., Kaur, J., Khatri, M., 2019. Agrochemical loaded biocompatible chitosan nanoparticles for insect pest management. *Biocatal. Agric. Biotechnol.* 18 <https://doi.org/10.1016/j.bcab.2019.101079>.
- Singh, H., Sharma, A., Bhardwaj, S.K., Arya, S.K., Bhardwaj, N., Khatri, M., 2021a. Recent advances in the applications of nano-agrochemicals for sustainable agricultural development. *Environ Sci Process Impacts.* <https://doi.org/10.1039/d0em00404a>.
- Singh, T.A., Sharma, A., Tejwan, N., Ghosh, N., Das, J., Sil, P.C., 2021b. A state of the art review on the synthesis, antibacterial, antioxidant, antidiabetic and tissue regeneration activities of zinc oxide nanoparticles. *Adv. Colloid Interf. Sci.* 295, 102495 <https://doi.org/10.1016/j.CIS.2021.102495>.
- Skalny, A.V., Rink, L., Ajsuvakova, O.P., Aschner, M., Gritsenko, V.A., Alekseenko, S.I., Svistunov, A.A., Petrakis, D., Spandidos, D.A., Aaseth, J., Tsatsakis, A., Tinkov, A.A., 2020. Zinc and respiratory tract infections: perspectives for COVID-19 (review). *Int. J. Mol. Med.* 46, 17–26. <https://doi.org/10.3892/IJMM.2020.4575>.
- Software, undefined C., 2020. The Jamovi Project (2020) Undefined-Undefined.
- Song, W., Jeong, M., Choi, D., Kim, K., Wie, M., 2019. Zinc oxide nanoparticles induce autophagy and apoptosis via oxidative injury and pro-inflammatory cytokines in primary astrocyte cultures. *Nanomater. (Basel, Switzerland)* 9. <https://doi.org/10.3390/NANO9071043>.
- Sudhakaran, S., Athira, S., Mohanan, P., 2019. Zinc oxide nanoparticle induced neurotoxic potential upon interaction with primary astrocytes. *Neurotoxicology* 73, 213–227. <https://doi.org/10.1016/J.NEURO.2019.04.008>.
- Tangirala, S., 2020. Evaluating the impact of GINI index and information gain on classification using decision tree classifier algorithm*. *IJACSA. Int. J. Adv. Comput. Sci. Appl.* 11.
- Team, C.R., others, undefined, Team, undefined R.C., 2013. R: A Language and Environment for Statistical Computing Undefined-Undefined.
- Titma, T., Shimmo, R., Siigur, J., Kahru, A., 2016. Toxicity of antimony, copper, cobalt, manganese, titanium and zinc oxide nanoparticles for the alveolar and intestinal epithelial barrier cells in vitro. *Cytotechnology* 68, 2363–2377. <https://doi.org/10.1007/S10616-016-0032-9>.
- Tolliver, L., Holl, N., Hou, F., Lee, H., Cambre, M., Huang, Y., 2020. Differential cytotoxicity induced by transition metal oxide nanoparticles is a function of cell killing and suppression of cell proliferation. *Int. J. Mol. Sci.* 21 <https://doi.org/10.3390/IJMS21051731>.
- Tripathy, N., Hong, T.K., Ha, K.T., Jeong, H.S., Hahn, Y.B., 2014. Effect of ZnO nanoparticles aggregation on the toxicity in RAW 264.7 murine macrophage. *J. Hazard. Mater.* 270, 110–117. <https://doi.org/10.1016/j.jhazmat.2014.01.043>.
- Vallabani, N., Sengupta, S., Shukla, R., Kumar, A., 2019. ZnO nanoparticles-associated mitochondrial stress- induced apoptosis and G2/M arrest in HaCaT cells: a mechanistic approach. *Mutagenesis* 34, 265–277. <https://doi.org/10.1093/MUTAGE/GEZ017>.
- Van Houwelingen, H.C., Zwinderman, K.H., Stijnen, T., 1993. A bivariate approach to meta-analysis. *Stat. Med.* 12, 2273–2284. <https://doi.org/10.1002/sim.4780122405>.
- Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor. *J. Stat. Softw.* 36, 1–48. <https://doi.org/10.18637/jss.v036.i03>.
- Vyslouzil, J., Kulich, P., Zeman, T., Vaculovic, T., Tvrdonová, M., Mikuška, P., Večeřa, Z., Stráská, J., Moravec, P., Balcar, V.J., Šerý, O., 2020. Subchronic continuous inhalation exposure to zinc oxide nanoparticles induces pulmonary cell response in mice. *J. Trace Elem. Med. Biol.* 61, 0–9. <https://doi.org/10.1016/j.jtemb.2020.126511>.
- Wang, C., Hu, X., Gao, Y., Ji, Y., 2015. ZnO nanoparticles treatment induces apoptosis by increasing intracellular ROS levels in LTP-2 cells. *Biomed. Res. Int.* 2015 <https://doi.org/10.1155/2015/423287>.
- Wang, J., Gao, S., Wang, S., Xu, Z., Wei, L., 2018a. Zinc oxide nanoparticles induce toxicity in CAL 27 oral cancer cell lines by activating PINK1/Parkin-mediated mitophagy. *Int. J. Nanomedicine* 13, 3441–3450. <https://doi.org/10.2147/IJN.S165699>.
- Wang, M., Yang, Q., Long, J., Ding, Y., Zou, X., Liao, G., Cao, Y., 2018b. A comparative study of toxicity of TiO₂, ZnO, and ag nanoparticles to human aortic smooth-muscle cells. *Int. J. Nanomedicine* 13, 8037. <https://doi.org/10.2147/IJN.S188175>.
- Wang, L., Guo, D., Wang, Z., Yin, X., Wei, H., Hu, W., Chen, R., Chen, C., 2020a. Zinc oxide nanoparticles induce human tenon fibroblast apoptosis through reactive oxygen species and caspase signaling pathway. *Arch. Biochem. Biophys.* 683 <https://doi.org/10.1016/J.ABB.2020.108324>.
- Wang, S., Lee, C., Lin, M., Chi, C., Chen, Y., Wang, G., Liao, K., Chiu, L., Wu, S., Huang, D., Chen, L., Shen, Y., 2020b. ZnO nanoparticles induced caspase-dependent apoptosis in gingival squamous cell carcinoma through mitochondrial dysfunction and p70S6K signaling pathway. *Int. J. Mol. Sci.* 21 <https://doi.org/10.3390/IJMS21051612>.
- Wu, Z., Yang, H., Archana, G., Rakshit, M., Ng, K., Tay, C., 2018. Human keratinocytes adapt to ZnO nanoparticles induced toxicity via complex paracrine crosstalk and Nrf2-proteasomal signal transduction. *Neurotoxicology* 12, 1215–1229. <https://doi.org/10.1080/17435390.2018.1537409>.
- Yan, Z., Xu, L., Han, J., Wu, Y.-J., Wang, W., Yao, W., Wu, W., 2014. Transcriptional and posttranscriptional regulation and endocytosis were involved in zinc oxide nanoparticle-induced interleukin-8 overexpression in human bronchial epithelial cells. *Cell Biol. Toxicol.* 79–88. <https://doi.org/10.1007/S10565-014-9270-9>, 2014 302 30.
- Yan, Y., Wang, G., Huang, J., Zhang, Y., Cheng, X., Chuai, M., Brand-Saberi, B., Chen, G., Jiang, X., Yang, X., 2020. Zinc oxide nanoparticles exposure-induced oxidative stress restricts cranial neural crest development during chicken embryogenesis. *Ecotoxicol. Environ. Saf.* 194 <https://doi.org/10.1016/J.ECOENV.2020.110415>.
- Yin, H., Casey, P.S., 2015. Effects of aspect ratio (AR) and specific surface area (SSA) on cytotoxicity and phototoxicity of ZnO nanomaterials. *Chemosphere* 124, 116–121. <https://doi.org/10.1016/j.chemosphere.2014.11.076>.
- Zhang, J., Qin, X., Wang, B., Xu, G., Qin, Z., Wang, J., Wu, L., Ju, X., Bose, D.D., Qiu, F., Zhou, H., Zou, Z., 2017. Zinc oxide nanoparticles harness autophagy to induce cell death in lung epithelial cells. *Cell Death Dis.* <https://doi.org/10.1038/cddis.2017.337>, 2017 87 8, e2954-e2954.
- Zinc Oxide Market Global Forecast to 2024 | MarketsandMarkets [WWW Document], n. d. URL <https://www.marketsandmarkets.com/Market-Reports/zinc-oxide-market-116672256.html> (accessed 5.15.21).