HHS Public Access

Author manuscript

Wiley Interdiscip Rev Nanomed Nanobiotechnol. Author manuscript; available in PMC
2019 July 01.

Published in final edited form as:

Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2018 July ; 10(4): e1507. doi:10.1002/wnan.1507.

***In vitro* to *in vivo* Benchmark Dose Comparisons to Inform Risk Assessment of Quantum Dot Nanomaterials**

Brittany A. Weldon,

Institute for Risk Analysis and Risk Communication, University of Washington, Seattle, WA

Department of Environmental and Occupational Health Sciences, University of Washington,
Seattle, WA

William C. Griffith,

Institute for Risk Analysis and Risk Communication, University of Washington, Seattle, WA

Department of Environmental and Occupational Health Sciences, University of Washington,
Seattle, WA

Tomomi Workman,

Institute for Risk Analysis and Risk Communication, University of Washington, Seattle, WA

Department of Environmental and Occupational Health Sciences, University of Washington,
Seattle, WA

David K. Scoville,

Center for Exposures, Diseases, Genomics and Environment, University of Washington, Seattle,
WA

Department of Environmental and Occupational Health Sciences, University of Washington,
Seattle, WA

Terrance J. Kavanagh, and

Center for Exposures, Diseases, Genomics and Environment, University of Washington, Seattle,
WA

Department of Environmental and Occupational Health Sciences, University of Washington,
Seattle, WA

Elaine M. Faustman

Institute for Risk Analysis and Risk Communication, University of Washington, Seattle, WA

Department of Environmental and Occupational Health Sciences, University of Washington,
Seattle, WA

Correspondence to: Elaine M. Faustman.

Brittany A. Weldon, No conflicts of interest

William C. Griffith, No conflicts of interest

Tomomi Workman, No conflicts of interest

David K. Scoville, No conflicts of interest

Terrance J. Kavanagh, No conflicts of interest

Elaine M. Faustman, No conflicts of interest

Abstract

Engineered nanomaterials are currently under review for their potential toxicity; however, their use in consumer/commercial products has continued to outpace risk assessments. *In vitro* methods may be utilized as tools to improve the efficiency of risk assessment approaches. We propose a framework to compare relationships between previously published *in vitro* and *in vivo* toxicity assessments of cadmium-selenium containing quantum dots (QDs) using benchmark dose (BMD) and dosimetric assessment methods. Although data were limited this approach was useful for identifying sensitive assays and strains. *In vitro* studies assessed effects of QDs in three pulmonary cell types across two mouse strains. Significant dose-response effects were modeled and a standardized method of BMD analysis was performed as a function of both exposure dose and dosimetric dose. *In vivo* studies assessed pulmonary effects of QD exposure across eight mouse strains. BMD analysis served as a basis for relative comparison with *in vitro* studies. We found consistent responses in common endpoints between *in vitro* and *in vivo* studies. Strain sensitivity was consistent between *in vitro* and *in vivo* studies, showing A/J mice more sensitive to QDs. Cell types were found to differentially take up QDs. Dosimetric adjustments identified similar sensitivity among cell types. Thus, BMD analysis can be used as an effective tool to compare the sensitivity of different strains, cell types, and assays to QDs. These methods allow for *in vitro* assays to be used to predict *in vivo* responses, improve the efficiency of *in vivo* studies, and allow for prioritization of nanomaterial assessments.

Graphical/Visual Abstract and Caption

Benchmark dose analysis can be used to compare effects of quantum dots in vitro to predict sensitivity of cell types, assays, and strains. Dosimetry was found to be critical for both in vitro and in vivo comparisons.

Introduction

Recent technological developments have employed the use of a wide array of nano-scaled materials (defined as having at least one dimension <100 nm), referred to generally as Engineered Nanomaterials (ENMs), for their attractive qualities including small size, large surface area, charge, optical qualities, strength and lightness of weight. ENMs are being used increasingly in medicine, manufacturing, and industry (Azzazy et al., 2007; Dabbousi et al., 1997). Applications of ENMs continue to accelerate, however the ability to fully characterize the risks associated with human exposures to nanomaterials has been somewhat limited.

Risk assessments of environmental health hazards have long been dependent on *in vivo* toxicity testing to determine acceptable levels of exposure to toxicants. This approach can require extensive testing in animals with high costs and time requirements. *In vitro* methods can lower some of these time and financial burdens by providing more rapid, high throughput assessments that can inform *in vivo* effects. The National Research Council (NRC) has outlined a vision of improved toxicity testing for efficiency and pathway-specific assessments (NRC, 2007). These methods rely heavily on linking toxicity assessments across biological contexts including linking *in vitro* to *in vivo* animal studies, and *in vivo* animal studies to effects in humans.

Recent efforts have been made by the United States Environmental Protection Agency (EPA) and the European Chemicals Agency (ECHA) to promote the use of *in vitro* toxicity testing of chemicals and other toxicants to improve the efficiency of toxicological assessments. EPA and the National Institute of Health's (NIH) Toxicology in the 21st Century (Tox21) goals for increasing high throughput testing promote the use of *in vitro* models for predictive toxicity testing. In addition, recent efforts by EPA to reform the Toxic Substances Control Act (TSCA) through the Frank R. Lautenberg Chemical Safety for the 21st Century Act promotes the use of non-animal testing and other *in vitro* and *in silico* techniques (USEPA, 2016). The European Chemicals Agency (ECHA) has employed these same principles in their REACH legislation that supports the use of alternative testing methods for improved efficiency and reduced animal testing (EU, 2006). In order to better utilize *in vitro* studies in risk assessment, tools are needed to translate *in vitro* findings to predict *in vivo* results for the reduction and refinement of *in vivo* studies.

Likewise, the rapid pace of development in nanotechnology creates an urgent need for improved efficiency in risk assessment. While some *in vitro* studies have been shown to be predictive of *in vivo* effects for some chemicals (Browne et al., 2015), the unique properties of ENMs may affect *in vitro* behaviour (Teeguarden et al., 2007). As such, there has been some question as to the utility of the sole use of *in vitro* screening for toxicity of nanoparticles (Donaldson et al., 2009). An EPA Nanotechnology Workgroup identified the need to determine whether current testing methods for chemical hazards, such as the use of

in vitro studies, are appropriate for evaluation of hazard of nanomaterials for risk assessment (USEPA, 2007).

To begin to address this question, the goal of our study was to investigate the relative relationships between *in vitro* and *in vivo* findings using poly(maleic anhydride-alt-1-tetradecene), tri-n-octylphosphine oxide (PMAT-TOPO) co-polymer coated CdSe core/ZnS shell composite quantum dots (QDs), a commonly used inorganic ENM, as a case study. We developed a benchmark dose (BMD)-based model for assessing sensitivity of multiple cell types, strains and assays to QD exposure by common endpoints between test systems. We hypothesized that BMD analysis may serve as a tool to characterize relative sensitivity of *in vitro* endpoints, cell types, and strains. We propose that this method can serve as an effective tool to translate *in vitro* sensitivity to better inform *in vivo* studies for the hazard identification and risk characterization of ENMs in risk assessments.

MATERIALS AND METHODS

To investigate the relationships between *in vitro* and *in vivo* findings, we accessed previously published data made available through the NIEHS Chemical Effects in Biological Systems (CEBS) database repository from NCNHIR Centers. *In vitro* investigations of PMAT-TOPO coated CdSe/ZnS quantum dots (QDs) were reported by Lee et al. (2015) and *in vivo* studies of QD effects in mice are as previously reported (Scoville et al., 2015).

In vitro study methods

Briefly, Lee et al. investigated TOPO-PMAT-coated QDs in three murine cell types including bone marrow derived macrophages (BMDM), alveolar macrophages (AM) obtained from *in vivo* bronchoalveolar lavage, and mouse tracheal epithelial cells (MTECs) cultured *in vitro*. Effects in these cell types were investigated in two mouse strains, C57BL/6J and A/J. MTECs were differentiated as organotypic monolayers at an air-liquid interface which contain several cell types (e.g. ciliated epithelial cells, mucus secreting goblet cells). BMDMs were cultured in growth factor-containing medium for 7 days, while AMs were obtained via bronchoalveolar lavage from sacrificed mice. Cells were exposed to 0, 10, 40, 80, or 100 nM QDs for 24 hours. Uptake of Cd, cytotoxicity, and proinflammatory cytokine expression by relative quantification (RQ) of their mRNAs were assessed. Results showed dose-dependent increases in Cd uptake into all cell types in both strains when analyzed by inductively coupled plasma-mass spectrometry (ICP-MS). BMDMs and AMs were observed to take up greater amounts of Cd compared to MTECs. No significant dose-response was observed in cytotoxicity assessments. QDs were observed to induce a dose-dependent increase in levels of CXCL1 (CXC Motif Chemokine Ligand 1, also known as KC) and IL6 (Interleukin 6) neutrophil recruiting cytokines in A/J BMDM and AM cultures. MTECs showed a significant QD dose response for expression of CXCL1.

In vivo study methods

Scoville and co-workers (Scoville et al., 2015) performed an *in vivo* investigation of TOPO-PMAT-coated QD effects in the eight genetically diverse founder mouse strains of the Collaborative Cross (Churchill et al., 2004). Animals were exposed to 0 or 6 µg Cd

equivalents/kg body weight of 10 nM QDs by oropharyngeal aspiration and sacrificed 8 hours after exposure. Bronchoalveolar lavage (BAL) was performed at the time of sacrifice and BAL fluid (BALF) was assessed for neutrophil infiltration and proinflammatory cytokine protein levels. Tissue cadmium dosimetry was assessed via ICP-MS and total glutathione in lung tissue was determined. QDs were found to induce variable inflammatory responses among the mouse strains. Significant neutrophil infiltration was found in CAST and NZO strains, while the A/J strain was found to have significant increases in cytokines assessed in BALF including CXCL1, MIP1 α , MIP1 γ , and G-CSF. In a sensitivity analysis where potential outliers were removed, A/J mice also had significantly increased neutrophil infiltration.

Benchmark dose modeling methods

Dose Response modeling—In order to compare toxicity responses observed in these datasets, we first established fitness criteria for dose response and benchmark dose modeling. Fitness criteria were based on a modified version of EPA's framework for BMD modeling and technical guidance (USEPA, 2012). The criteria were modified because only a single dose was available or suitable in the studies. For regulatory purposes more doses are required but the criteria were modified to use the limited data to guide the design of future studies that would be suitable for regulatory purposes. From these criteria, studies were limited to those showing significant dose-response effects. We modeled dose response curves for *in vitro* (Lee et al., 2015) and *in vivo* (Scoville et al., 2015) endpoints that fit these conditions. *In vitro* assessments included mRNA expression of proinflammatory markers IL6 and CXCL1 in cells from A/J and C57BL/6J mice. *In vivo* assessments included proinflammatory marker CXCL1 and neutrophil infiltration in BALF across eight mouse strains.

Data sets that did not pass fitness criteria (e.g. lack of significant dose-response) were excluded from analysis. For *in vitro* assessments that showed very steep responses, we utilized the initial cytokine response that defined the curve (first dose). Only the initial cytokine response was used for *in vitro* studies as higher doses showed a decreasing trend with dose which was not amenable to model fits and calculations of BMDs. Overall, linear dose response models were used since the only available values were for controls and a single dose for both the *in vitro* data and the *in vivo* data. If data had been available at additional doses other types of models would have been considered. The linear dose response models were used to identify which future studies should be conducted with additional doses from which BMDs could be calculated for regulatory purposes.

From the dose response models, we calculated benchmark doses using EPA's Benchmark Dose Software following a modified version of EPA's framework for BMD modelling (USEPA, 2012). When calculating a BMD, a standardized benchmark response level (BMR) was used to calculate the dose at which a significant response will occur (Kavlock et al., 1995; Wignall et al., 2014). A BMR of 1 standard deviation change in response relative to controls was used for *in vitro* endpoints and *in vivo* cytokine endpoints (Davis et al., 2011; Meyer et al., 2012). A BMR of 1 standard deviation change in response relative to the mean

of the control is approximately equivalent to a 10% change in response relative to the control mean (Crump, 1995).

All BMDs were calculated and compared by nominal exposure dose and by dosimetric dose of Cd (from QDs) in cells. Exposure dose was defined as the concentration of QDs in exposure media at the time of treatment (nM QDs), while dosimetric dose is defined as the amount of Cd measured in or associated with cells at the time of toxicity assessment (ng Cd/100,000 cells).

Relative comparisons between in vitro and in vivo—*In vitro* benchmark doses were used as a basis for comparison of sensitivity of *in vitro* and *in vivo* systems to QD exposure by both nominal (exposure) dose as well as by dosimetric dose. BMDs with common dose units, nM QDs or ng Cd/100,000 cells, allowed for the direct comparison between endpoints, cell types, and strains. Sensitivity was compared across test assay, cell type, and strain, where sensitivity was defined as having a lower BMD.

We used R statistical software to visualize BMD relationships among varying test conditions (RCoreTeam, 2015). Sensitive assays and strains identified in *in vitro* comparisons were compared with *in vivo* findings for consistency in results. *In vitro* assays were considered predictive if sensitivity was identified among common endpoints consistently between *in vitro* and *in vivo* studies.

Significance tests between BMDs—Statistical significance between benchmark doses was determined by calculating standard error and p-values based on asymptotic normality and confidence intervals at BMDs (Supplemental information, equations 1 and 2).

RESULTS

Data collection and suitability for benchmark dose modeling

We accessed previously published data from *in vitro* investigations of QD toxicity from Lee and co-workers (Lee et al., 2015) and *in vivo* studies of effects in mice from Scoville and colleagues (Scoville et al., 2015). Endpoints assessed following *in vitro* QD exposure included cytotoxicity and pro-inflammatory responses in three cell types. Of these endpoints, pro-inflammatory responses CXCL1 and IL6 in all cell types passed the established fitness criteria, while no significant dose-response was observed for other endpoints in the study such as cytotoxicity. Of the responses assessed *in vivo*, pro-inflammatory responses CXCL1 and neutrophils in BALF passed fitness criteria and were modeled for comparison with corresponding *in vitro* assessments. Because data from controls and single doses were used, all endpoints were fit to a linear model.

Comparison of BMDs in in vitro assessments

We calculated benchmark doses (BMDs) for exposure dose and dosimetric dose for two *in vitro* pro-inflammatory marker (IL6 and CXCL1) endpoints and compared BMDs between cell types, strains, and endpoints. A factor was considered more sensitive compared to another factor if its BMD was significantly lower than the other factor.

Exposure dose comparison—Table 1 shows BMDs and BMDLs (lower 95% confidence limit of the BMD) for each *in vitro* endpoint by exposure dose (second column from right). MTECs were significantly less sensitive to QDs than AMs and BMDMs for both endpoints in both strains ($p < 0.05$). AMs and BMDMs showed similar BMDs within each endpoint in the C57BL/6J strain ($p > 0.05$), while there were significant differences in sensitivity between these cell types in the A/J strain ($p < 0.05$). Overall, the A/J strain was significantly more sensitive to QDs in the CXCL1 endpoint for all cell types ($p < 0.05$), and slightly more sensitive in the IL6 assessment for BMDMs ($p = 0.058$). AMs and BMDMs were similar in sensitivity for IL6 and CXCL1 assays in both strains ($p > 0.05$).

Dosimetric dose comparison—When comparing BMDs by dosimetric dose (Table 1, rightmost column), MTECs were generally observed to be equally as sensitive as AMs and BMDMs in both strains and both endpoints ($p > 0.05$, $p > 0.05$, respectively). The A/J strain was observed to be more sensitive by all cell types compared to the C57BL/6J strain in the CXCL1 endpoint ($p < 0.05$). The CXCL1 assay was a more sensitive endpoint in MTECs in both strains compared to the IL6 assay. IL6 and CXCL1 assays were similarly sensitive for both AMs and BMDMs across both strains ($p > 0.05$).

Exposure dose and dosimetric dose BMD comparisons—BMD and BMDL relationships by exposure dose and dosimetric dose were compared among cell type, strain, and proinflammatory cytokine expression in *in vitro* assays (Figure 1). BMD analysis of CXCL1 and IL6 expression by exposure dose (exposure concentration, Figure 1A) shows BMDs and BMDLs for MTECs to be higher than those for BMDMs ($p < 0.05$) and AMs ($p < 0.05$) by both endpoints, with no overlap in confidence limits of the BMD (BMDL, or the BMD of the 95% confidence limit). This suggests that MTECs may be less sensitive to QDs by one to two orders of magnitude across both strains and endpoints. AMs and BMDMs were similarly sensitive to QDs in the C57BL/6J strain ($p > 0.05$). BMDs for AMs and BMDMs were significantly different in the A/J strain ($p < 0.05$) however, BMDs and BMDLs were generally within one order of magnitude in both endpoints.

In contrast, when assessing the same responses as a function of dosimetric dose (Figure 1B), we observed that the MTECs were generally equally sensitive as BMDMs ($p > 0.05$) and AMs ($p > 0.05$) overall, as indicated by similar BMD values and a high degree of overlap of confidence limits. AMs and BMDMs were similarly sensitive to each other within each endpoint and strain in both exposure dose and dosimetric assessments. The relative relationships between AMs and BMDMs remained consistent between the two assessments, while the MTEC shows a higher degree of sensitivity when assessing BMDs by dosimetry.

In vivo results

Scoville et al. (2015) quantified the protein expression of CXCL1 in BALF of eight mouse strains after *in vivo* exposure to QDs (Figure 2). CXCL1 expression was significant in the A/J, NOD, and NZO strains, with A/J ranking as the most sensitive. A significant amount of genetic variability in CXCL1 expression response was observed among strains. For example, A/J mice showed about two times more CXCL1 after exposure to QDs compared to strain-specific controls than C57BL/6J mice. In addition, there was a large amount of genetic

variability in control response where controls in the A/J strain showed about twice as much CXCL1 in BALF as controls of the C57BL/6J strain. Significant responses were not observed in C57BL/6J, 129S1, PWK, WSB, or CAST strains compared to strain specific control values.

Dose responses were modeled for significant *in vivo* endpoints (Scoville et al., 2015) including CXCL1 protein expression and percent neutrophil infiltration in BALF. BMDs were calculated by exposure dose ($\mu\text{g Cd equivalents/kg body weight}$) and by dosimetric dose (ng Cd/kg tissue) and are shown in Table 2. The 129S1, C57BL/6J, CAST, PWK, and WSB strains did not show significant dose responses by either of these endpoints, and thus BMDs were not calculated. A/J was the most sensitive strain for the CXCL1 endpoint, for both the exposure dose and dosimetric dose. Both endpoints were similarly sensitive indicators of response.

Exposure dose and dosimetric dose comparison—Comparison of responses across strains *in vivo* shows the A/J strain has the lowest BMDs by both exposure dose and by dosimetric dose (Table 2), indicating the A/J strain is the most sensitive strain by these endpoints. When corrected for dosimetry, the A/J strain was statistically significantly lower than both the NOD and NZO strains ($p < 0.05$). The NOD and NZO strains were not significantly different from each other by either endpoint, both by exposure dose and dosimetric dose assessments ($p > 0.05$). Significant dose response effects were not observed in several of the strains including C57BL/6J, 129S1, PWK, WSB, and CAST.

Comparison across model systems (in vitro and in vivo)

We found the A/J strain was the more sensitive to QD exposure than the C57BL/6J both *in vitro* and *in vivo*. The A/J strain was more sensitive in both the IL6 and CXCL1 assays *in vitro* as well as in the CXCL1 and neutrophil assays *in vivo*.

DISCUSSION

The risk assessment framework utilizes *in vitro* studies as part of hazard identification to identify possible mechanisms of toxicity and inform potential endpoints of toxicity *in vivo*. Risk characterization however, depends heavily on the use of *in vivo* studies that can be time and resource intensive. The state of the field of toxicology in the 21st century and beyond requires risk assessment methods that are efficient and lean processes (EU, 2006; NRC, 2007). *In vitro* research has been proposed as an inexpensive and more efficient alternative to replace, reduce, and refine *in vivo* toxicity assessments (Nel et al., 2006; Zurlo et al., 1996).

In this study, we hypothesized that *in vitro* toxicity assessments of engineered nanomaterials can be useful in better informing and refining *in vivo* studies for risk assessment. We used standardized BMD analysis as a tool to identify *in vitro* endpoints and test systems that are most sensitive to QDs. Similarly, Wignall and colleagues (Wignall et al., 2014) used a standardized BMD approach to compare across many environmental chemicals for investigation of effects across various studies and chemicals.

We found that the A/J mouse strain was more sensitive to QD exposure than the C57BL/6J mouse strain consistently in both *in vitro* and *in vivo* using common endpoints. This effect was observed when assessing by both nominal and dosimetry dose metrics. Strain sensitivity is a critical consideration for *in vivo* assessments for hazard identification of QDs and other inorganic ENMs. The C57BL/6J mouse strain is one of the most frequently used inbred strains in toxicological assessments of nanoparticles and other chemicals (JAXLabs, 2016; Zurita et al., 2011). Our findings suggest that studies investigating effects of nanoparticles in solely the C57BL/6J strain may overlook effect levels in other genetic backgrounds. The genetic differences among the eight founder strains represents approximately 90% of murine genetic variability and captures a similar distribution of allele frequencies as humans (Churchill et al., 2004; Roberts et al., 2007). As such, capturing this variability can better inform risk assessments of potential effect levels in sensitive populations.

While BMD analysis by exposure dose showed MTECs were less sensitive to QDs than AMs and BMDMs, analysis by dosimetry showed that MTECs were generally equally as sensitive to QD exposure, in general. MTECs were observed to take up less Cd (QDs) per nominal exposure than AMs and BMDMs, however their proinflammatory cytokine response is equally as pronounced per amount of Cd, indicating that dosimetry is critical in the consideration of ENM toxicity *in vitro* for comparison of cell types. This observation of difference in uptake between cell type may be due to the differing biological functions of cell types as AMs and BMDMs are phagocytic cell types and have been shown to take up greater amounts of ENMs including QDs (Zhang et al., 2013). In contrast, MTECs are epithelial cells whose primary biological functions do not necessarily include phagocytizing particles or other foreign materials. Also, MTECs are organotypic cell cultures grown and differentiated at an air-liquid interface and may be more similar to cells *in vivo* than AMs and BMDMs. Similar findings in sensitivity among cell types may be used to inform future *in vitro* work and promote studies in cell types that are faster and less resource intensive to yield similar results.

Measuring dosimetry allows for the assessment of toxicity of particles based on delivery of material to the cell culture following an exposure. This approach is based on the assumption and basic tenet of toxicology that effects observed are caused by the material delivered to, and thus able to interact with the receptor (cell or tissue, etc.). Assessing *in vitro* effects as a function of dosimetric dose creates a common denominator by which different ENMs, assays, cell types, strains, and culture conditions can be compared (Liu et al., 2015; Teeguarden et al., 2007). Because dosimetry can also be measured *in vivo*, *in vitro* findings as a function of dosimetry can be translated to effects in similar cell types *in vivo*. This is especially relevant for organotypic cell culture models, such as MTECs, where cultures more closely represent tissue structure and function, and delivery of particles to culture systems more closely represents *in vivo* exposure kinetics (Weber et al., 2016).

Unlike many chemicals, *in vitro* investigation of ENMs pose unique challenges relating to physicochemical properties of the materials. Particles in culture media have a propensity to aggregate or agglomerate, significantly affecting the delivery rate and contact of particles to the cells in culture (Teeguarden et al., 2007). Dissolution, or the dissolving of ions from soluble or semi-soluble particles in solution, is influenced by particle physicochemical

characteristics as well as local culture conditions (Shannahan et al., 2013; Utembe et al., 2015). The formation of a protein corona, or protein layer around ENMs in media, may affect the uptake and toxicity observed in culture (Chandran et al., 2017; Choi et al., 2017; Monteiro-Riviere et al., 2013; Shannahan et al., 2016; Shannahan et al., 2015; Shannahan et al., 2015). Slight differences in particle physicochemical properties (e.g., size or stabilizing coating) and culture conditions (e.g., pH or presence of protein in media) may result in differing extent of effects on cells in culture. Measuring the dosimetry of materials in cells after exposure allows for the direct comparison between culture systems.

We observed consistency in response by common endpoints in both *in vitro* and *in vivo* studies. Assessments of the BMDs by the proinflammatory marker CXCL1 were found to be sensitive endpoints in both test systems. The CXCL1 endpoint also showed the A/J strain was more sensitive than the C57BL/6J strain both *in vitro* and *in vivo*. CXCL1 plays a critical role in neutrophil recruitment to sites of injury and inflammation, and was found to be highly correlated with neutrophil presence in the airway following exposure (Scoville et al., 2015). Acute lung injury has been shown to initiate proinflammatory cytokines such as CXCL1 and quickly progress into fibrotic responses (Wynn, 2011). Both macrophage and airway epithelial cell types used in these studies have been associated with the development of pathologies such as pulmonary fibrosis. Several mechanistic hypotheses describe how environmental insults such as exposure to ENMs can activate neutrophils and other cell types to contribute proinflammatory factors that can go on to activate interstitial fibroblasts and eventually lead to fibrosis (Bringardner et al., 2008). Moreover, airway epithelial cells have been described to contribute to this process by proliferating and transitioning into microfibroblasts or fibroblasts (Kim et al., 2006).

Our analysis showed dosimetry was critical to identify cell type sensitivity and strain sensitivity in both *in vitro* and *in vivo* systems. Because dosimetry of inorganic nanomaterials can be easily quantified *in vitro* and *in vivo* using ICP-MS and other metals quantification methods, this framework for the assessment of *in vitro* ENM studies to inform *in vivo* studies and risk assessment may be applied to other inorganic nanomaterials, such as silver nanoparticles (AgNPs). AgNPs are increasingly being used in consumer and commercial products for their antimicrobial activity and have been actively studied for potential health effects using both *in vitro* and *in vivo* studies.

While this framework of utilizing BMD analysis and dosimetry-based *in vitro* studies can provide insights useful in informing *in vivo* studies and ENM risk assessment, several limitations of this work are noted. First, the database available provided data that was limited to controls and a single dose. Given the experimental nature of ENM assessment, our BMD analysis focused on the area of the dose response curve where significant change was observed. As such, our dose-response analyses were constrained to controls and a single dose both *in vivo* and *in vitro*. The addition of further lower doses that would show an increasing response across multiple doses could better represent the dose-response effect and BMD. In addition, interpretation of *in vivo* data was limited as the dosimetric Cd measures were taken 8 hours after exposure. As such, the Cd measured may not represent the original dose to the tissue, rather represents the residual Cd after exposure. Differences in toxicokinetics and clearance among the strains may have affected this residual Cd level.

Next, a standardized benchmark response (BMR) of 1 standard deviation change relative to controls was used across all BMD analyses. This approach allowed for a consistent benchmark to compare across endpoints and has been utilized by other groups to make comparisons across endpoints (Wignall et al., 2014). Findings within this framework are best geared toward informing directions of *in vivo* research non-quantitatively. Finally, while A/J strain sensitivity relative to the C57BL/6J was identified both *in vitro* and *in vivo*, the *in vitro* dataset was limited to just these two strains. Further *in vitro* studies investigating sensitivity of effects in other strains may further substantiate these relationships.

Conclusion

In summary, we found that a standardized BMD and dosimetric analysis allows for the comparison of sensitivity of assay, cell type, and animal strains to QDs. We observed that when assessed by dosimetry, exposure dose findings of differential toxicity and inflammation by cell type are not significant. We observed significant mouse strain dependent heterogeneity in response to QDs. While C57BL/6J mice are commonly used in toxicology studies, they tend to be relatively resistant to QD (Scoville et al., 2015) and silver nanoparticle (Scoville et al., 2017) -induced pulmonary inflammation compared to the A/J strain. This suggests that assessment of the relative hazards of ENMs should include exposure modeling, toxicokinetic and toxicodynamic modeling, as well as gene/environment interactions. We found that when put into context, *in vitro* assays provide valuable insight on critical factors for ENM risk assessment approaches.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH Grants U19ES019545, P30ES007033, T32ES007032, T32ES015459 and EPA Grant RD-83573801. This presentation was developed under Assistance Agreement No. 83573801 awarded by the U.S. Environmental Protection Agency to Elaine M. Faustman. It has not been formally reviewed by EPA. The views expressed in this presentation are solely those of the authors and do not necessarily reflect those of the Agency. EPA does not endorse any products or commercial services mentioned in this publication.

We would like to thank the authors of the *in vitro* study referenced here (Lee et al., 2015) for their contributions and access to raw data for our analyses as well as the NIEHS Centers for Nanotechnology Health Implications Research (NCNHIR) Consortium for making these data available.

References

- Azzazy HM, Mansour MM, Kazmierczak SC. From diagnostics to therapy: prospects of quantum dots. Clin Biochem. 2007; 40:917–927. [PubMed: 17689518]
- Bringardner BD, Baran CP, Eubank TD, Marsh CB. The role of inflammation in the pathogenesis of idiopathic pulmonary fibrosis. Antioxidants & redox signaling. 2008; 10:287–301. [PubMed: 17961066]
- Browne P, Judson RS, Casey WM, Kleinstreuer NC, Thomas RS. Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model. Environmental science & technology. 2015; 49:8804–8814. [PubMed: 26066997]
- Chandran P, Riviere JE, Monteiro-Riviere NA. Surface chemistry of gold nanoparticles determines the biocorona composition impacting cellular uptake, toxicity and gene expression profiles in human endothelial cells. Nanotoxicology. 2017; 11:507–519. [PubMed: 28420299]

- Choi K, Riviere JE, Monteiro-Riviere NA. Protein corona modulation of hepatocyte uptake and molecular mechanisms of gold nanoparticle toxicity. *Nanotoxicology*. 2017; 11:64–75. [PubMed: 27885867]
- Churchill GA, Airey DC, Allayee H, Angel JM, Attie AD, Beatty J, Beavis WD, Belknap JK, Bennett B, Berrettini W, Bleich A, Bogue M, Broman KW, Buck KJ, Buckler E, Burmeister M, Chesler EJ, Cheverud JM, Clapcote S, Cook MN, Cox RD, Crabbe JC, Crusio WE, Darvasi A, Deschepper CF, Doerge RW, Farber CR, Forejt J, Gaile D, Garlow SJ, Geiger H, Gershenfeld H, Gordon T, Gu J, Gu W, de Haan G, Hayes NL, Heller C, Himmelbauer H, Hitzemann R, Hunter K, Hsu HC, Iraqi FA, Ivandic B, Jacob HJ, Jansen RC, Jepsen KJ, Johnson DK, Johnson TE, Kempermann G, Kendziorowski C, Kotb M, Kooy RF, Llamas B, Lammert F, Lassalle JM, Lowenstein PR, Lu L, Lusis A, Manly KF, Marcucio R, Matthews D, Medrano JF, Miller DR, Mittleman G, Mock BA, Mogil JS, Montagutelli X, Morahan G, Morris DG, Mott R, Nadeau JH, Nagase H, Nowakowski RS, O'Hara BF, Osadchuk AV, Page GP, Paigen B, Paigen K, Palmer AA, Pan HJ, Peltonen-Palotie L, Peirce J, Pomp D, Pravenec M, Prows DR, Qi Z, Reeves RH, Roder J, Rosen GD, Schadt EE, Schalkwyk LC, Seltzer Z, Shimomura K, Shou S, Sillanpaa MJ, Siracusa LD, Snoeck HW, Spearow JL, Svenson K, Tarantino LM, Threadgill D, Toth LA, Valdar W, de Villena FP, Warden C, Whatley S, Williams RW, Wiltshire T, Yi N, Zhang D, Zhang M, Zou F. The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nat Genet*. 2004; 36:1133–1137. [PubMed: 15514660]
- Crump KS. Calculation of Benchmark Doses from Continuous Data. *Risk Analysis*. 1995; 15:79–89.
- Dabbousi BO, Rodriguez-Viejo J, Mikulec FV, Heine JR, Mattoussi H, Ober R, Jensen KF, Bawendi MG. (CdSe)ZnS core-shell quantum dots: synthesis and characterization of a size series of highly luminescent nanocrystallites. *J. Phys. Chem.* 1997; 9463
- Davis JA, Gift JS, Zhao QJ. Introduction to benchmark dose methods and U.S. EPA's benchmark dose software (BMDS) version 2.1.1. *Toxicol Appl Pharmacol*. 2011; 254:181–191. [PubMed: 21034758]
- Donaldson K, Borm PJ, Castranova V, Gulumian M. The limits of testing particle-mediated oxidative stress in vitro in predicting diverse pathologies; relevance for testing of nanoparticles. *Particle and fibre toxicology*. 2009; 6:13. [PubMed: 19397808]
- EU. Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency. 2006
- JAXLabs. The Jackson Laboratory: C57BL/6J Mouse Strain Data Sheet. Available at: <https://www.jax.org/strain/000664>
- Kavlock RJ, Allen BC, Faustman EM, Kimmel CA. Dose-response assessments for developmental toxicity. IV. Benchmark doses for fetal weight changes. *Fundam Appl Toxicol*. 1995; 26:211–222. [PubMed: 7589910]
- Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN, Sheppard D, Chapman HA. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:13180–13185. [PubMed: 16924102]
- Lee V, McMahan RS, Hu X, Gao X, Faustman EM, Griffith WC, Kavanagh TJ, Eaton DL, McGuire JK, Parks WC. Amphiphilic polymer-coated CdSe/ZnS quantum dots induce pro-inflammatory cytokine expression in mouse lung epithelial cells and macrophages. *Nanotoxicology*. 2015; 9:336–343. [PubMed: 24983898]
- Liu R, Liu HH, Ji Z, Chang CH, Xia T, Nel AE, Cohen Y. Evaluation of Toxicity Ranking for Metal Oxide Nanoparticles via an in Vitro Dosimetry Model. *ACS Nano*. 2015; 9:9303–9313. [PubMed: 26284985]
- Meyer KC, Raghu G, Baughman RP, Brown KK, Costabel U, du Bois RM, Drent M, Haslam PL, Kim DS, Nagai S, Rottoli P, Saltini C, Selman M, Strange C, Wood B. American Thoracic Society Committee on BALiLD. An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. *Am J Respir Crit Care Med*. 2012; 185:1004–1014. [PubMed: 22550210]
- Monteiro-Riviere NA, Samberg ME, Oldenburg SJ, Riviere JE. Protein binding modulates the cellular uptake of silver nanoparticles into human cells: implications for in vitro to in vivo extrapolations? *Toxicol Lett*. 2013; 220:286–293. [PubMed: 23660336]

- Nel A, Xia T, Madler L, Li N. Toxic potential of materials at the nanolevel. *Science*. 2006; 311:622–627. [PubMed: 16456071]
- NRC. Toxicity Testing in the 21st Century: A Vision and a Strategy. 2007
- RCoreTeam. R: A language and environment for statistical computing. 2015. Available at: <https://www.r-project.org/>
- Roberts A, Pardo-Manuel de Villena F, Wang W, McMillan L, Threadgill DW. The polymorphism architecture of mouse genetic resources elucidated using genome-wide resequencing data: implications for QTL discovery and systems genetics. *Mamm Genome*. 2007; 18:473–481. [PubMed: 17674098]
- Scoville DK, Botta D, Galdanes K, Schmuck SC, White CC, Stapleton PL, Bammler TK, MacDonald JW, Altemeier WA, Hernandez M, Kleeberger SR, Chen LC, Gordon T, Kavanagh TJ. Genetic determinants of susceptibility to silver nanoparticle-induced acute lung inflammation in mice. *FASEB J*. 2017
- Scoville DK, White CC, Botta D, McConnachie LA, Zadworny ME, Schmuck SC, Hu X, Gao X, Yu J, Dills RL, Sheppard L, Delaney MA, Griffith WC, Beyer RP, Zangar RC, Pounds JG, Faustman EM, Kavanagh TJ. Susceptibility to quantum dot induced lung inflammation differs widely among the Collaborative Cross founder mouse strains. *Toxicol Appl Pharmacol*. 2015; 289:240–250. [PubMed: 26476918]
- Shannahan JH, Fritz KS, Raghavendra AJ, Podila R, Persaud I, Brown JM. From the Cover: Disease-Induced Disparities in Formation of the Nanoparticle-Biocorona and the Toxicological Consequences. *Toxicol Sci*. 2016; 152:406–416. [PubMed: 27255384]
- Shannahan JH, Lai X, Ke PC, Podila R, Brown JM, Witzmann FA. Silver nanoparticle protein corona composition in cell culture media. *PLoS One*. 2013; 8:e74001. [PubMed: 24040142]
- Shannahan JH, Podila R, Aldossari AA, Emerson H, Powell BA, Ke PC, Rao AM, Brown JM. Formation of a protein corona on silver nanoparticles mediates cellular toxicity via scavenger receptors. *Toxicol Sci*. 2015; 143:136–146. [PubMed: 25326241]
- Shannahan JH, Podila R, Brown JM. A hyperspectral and toxicological analysis of protein corona impact on silver nanoparticle properties, intracellular modifications, and macrophage activation. *Int J Nanomedicine*. 2015; 10:6509–6521. [PubMed: 26508856]
- Teeguarden JG, Hinderliter PM, Orr G, Thrall BD, Pounds JG. Particokinetics in vitro: dosimetry considerations for in vitro nanoparticle toxicity assessments. *Toxicol Sci*. 2007; 95:300–312. [PubMed: 17098817]
- USEPA. Science Policy Council Nanotechnology White Paper. 2007
- USEPA. Benchmark Dose Technical Guidance. 2012
- USEPA. H.R. 2576 — 114th Congress: Frank R. Lautenberg Chemical Safety for the 21st Century Act. 2016. Available at: <https://www.govtrack.us/congress/bills/114/hr2576>
- Utembe W, Potgieter K, Stefaniak AB, Gulumian M. Dissolution and biodegradability: Important parameters needed for risk assessment of nanomaterials. *Part Fibre Toxicol*. 2015; 12:11. [PubMed: 25927337]
- Weber EJ, Chapron A, Chapron BD, Voellinger JL, Lidberg KA, Yeung CK, Wang Z, Yamaura Y, Hailey DW, Neumann T, Shen DD, Thummel KE, Muczynski KA, Himmelfarb J, Kelly EJ. Development of a microphysiological model of human kidney proximal tubule function. *Kidney Int*. 2016; 90:627–637. [PubMed: 27521113]
- Wignall JA, Shapiro AJ, Wright FA, Woodruff TJ, Chiu WA, Guyton KZ, Rusyn I. Standardizing benchmark dose calculations to improve science-based decisions in human health assessments. *Environ Health Perspect*. 2014; 122:499–505. [PubMed: 24569956]
- Wynn TA. Integrating mechanisms of pulmonary fibrosis. *J Exp Med*. 2011; 208:1339–1350. [PubMed: 21727191]
- Zhang Y, Pan H, Zhang P, Gao N, Lin Y, Luo Z, Li P, Wang C, Liu L, Pang D, Cai L, Ma Y. Functionalized quantum dots induce proinflammatory responses in vitro: the role of terminal functional group-associated endocytic pathways. *Nanoscale*. 2013; 5:5919–5929. [PubMed: 23703357]

- Zurita E, Chagoyen M, Cantero M, Alonso R, Gonzalez-Neira A, Lopez-Jimenez A, Lopez-Moreno JA, Landel CP, Benitez J, Pazos F, Montoliu L. Genetic polymorphisms among C57BL/6 mouse inbred strains. *Transgenic Res.* 2011; 20:481–489. [PubMed: 20506040]
- Zurlo J, Rudacille D, Goldberg AM. The three Rs: the way forward. *Environ Health Perspect.* 1996; 104:878–880. [PubMed: 8875163]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.

BMD (circles) and BMDL (triangles) values for IL6 and CXCL1 responses following *in vitro* Cd QD exposure by exposure dose (A) and dosimetric dose (B). BMDs are shown for multiple cell types, two different rodent strains (vertical panels). AMs and BMDMs are similarly sensitive across all endpoints and dose metrics. MTECs were significantly less sensitive than AMs and BMDMs by exposure dose assessment (A), but equally sensitive when adjusted for dosimetry (B).

Figure 2.

KC (CXCL1) protein expression in BALF after oropharyngeal aspiration exposure to QDs across eight mouse strains (Scoville et al., 2015). Significant increases in KC expression was observed in the A/J, NOD, and NZO strains. The A/J strain showed the greatest increase in KC relative to controls while the C57BL/6J strain ranked last in sensitivity by this endpoint. A large amount of genetic variability was observed across strains in both treated and control animals.

Table 1

Summary of BMDs and BMDLs calculated from *in vitro* exposures (Lee et al., 2015) to CdSe/ZnS QDs. BMDs and BMDLs are calculated by exposure concentration (0 or 10 nM QDs) and by dosimetric dose (ng Cd/100,000 cells) for significant endpoints of response (IL6 and CXCL1) for A/J and C57BL/6J mice in three cell types. n=4 per dose group.

Endpoint	Strain, Species	Cell type	Dosimetric Dose (ng Cd/100,000 cells)	Exposure Conc. BMD (BMDL) (nM QDs)	Dosimetric Dose BMD (BMDL) (ng Cd/100,000 cells)
IL6	A/J mouse	AM	0.3, 25.55	0.58 (0.40)	1.45 (1.01)
		BMDM	0.3, 36.9	0.24 (0.17)	0.87 (0.60)
		MTEC	0.12, 1.02	27.54 (6.51)	2.48 (0.59)
IL6	C57BL/6J mouse	AM	0.3, 29.15	0.43 (0.30)	1.25 (0.87)
		BMDM	0.3, 36.0	0.40 (0.28)	1.42 (0.99)
		MTEC	0.12, 2.54	31.49 (6.72)	7.62 (1.63)
CXCL1	A/J mouse	AM	0.3, 25.55	0.10 (0.07)	0.26 (0.18)
		BMDM	0.3, 36.9	0.37 (0.26)	1.35 (0.94)
		MTEC	0.12, 1.02	2.84 (1.84)	0.26 (0.17)
CXCL1	C57BL/6J mouse	AM	0.3, 29.15	0.76 (0.53)	2.2 (1.52)
		BMDM	0.3, 36.0	0.89 (0.62)	3.17 (2.19)
		MTEC	0.12, 2.54	9.69 (4.40)	2.35 (1.07)

Table 2

Summary of BMDs and BMDLs calculated from Scoville et al. (2015) *in vivo* exposures to CdSe/ZnS QDs. BMDs and BMDLs are calculated by exposure concentration ($\mu\text{g Cd equivalents/ kg body weight}$) and by dosimetric dose (ng Cd/mg tissue) for significant endpoints of response. $n=6$ per exposure group.

Exposure Concentration ($\mu\text{g Cd equivalents/ kg body weight}$)	Endpoint	Strain	Dosimetric Dose (ng Cd/mg tissue)	Exposure Conc. BMD (BMDL) ($\mu\text{g Cd equivalents/ kg body weight}$)	Dosimetric Dose BMD (BMDL) (ng Cd/mg tissue)
0, 6	KC (CXCL1)	A/J	0.002, 0.165	2.87 (1.84)	0.08 (0.05)
		NOD	0.0, 0.252	4.34 (2.29)	0.18 (0.10)
		NZO	0.0, 0.335	3.61 (2.10)	0.20 (0.12)
		129S1	Not significant	Not significant	Not significant
		C57BL/6J	Not significant	Not significant	Not significant
		CAST	Not significant	Not significant	Not significant
		PWK	Not significant	Not significant	Not significant
		WSB	Not significant	Not significant	Not significant
0, 6	% Neutrophils in BALF	A/J	0.002, 0.165	5.45 (2.76)	0.15 (0.08)
		NOD	0.0, 0.252	2.50 (1.61)	0.11 (0.07)
		NZO	0.0, 0.335	3.33 (2.01)	0.19 (0.11)
		129S1	Not significant	Not significant	Not significant
		C57BL/6J	Not significant	Not significant	Not significant
		CAST	Not significant	Not significant	Not significant
		PWK	Not significant	Not significant	Not significant
		WSB	Not significant	Not significant	Not significant