Supplemental Material

Interlaboratory Evaluation of *in Vitro* Cytotoxicity and Inflammatory Responses to Engineered Nanomaterials: The NIEHS NanoGo Consortium

Tian Xia¹, Raymond F. Hamilton Jr², James C. Bonner³, Edward D. Crandall⁴, Alison Elder⁵, Farnoosh Fazlollahi⁴, Teri A. Girtsman², Kwang Kim⁴, Somenath Mitra⁶, Susana A. Ntim⁶, Galya Orr⁷, Mani Tagmont⁸, Alexia J. Taylor³, Donatello Telesca¹, Ana Tolic⁷, Christopher D. Vulpe⁸, Andrea J. Walker⁵, Xiang Wang¹, Frank A. Witzmann⁹, Nianqiang Wu¹⁰, Yumei Xie⁷, Jeffery I. Zink¹, Andre Nel¹, and Andrij Holian²

¹Department of Medicine, Division of NanoMedicine, Center for Environmental Implications of Nanotechnology, California Nanosystems Institute, University of California at Los Angeles, Los Angeles, California, USA
²Center for Environmental Health Sciences, Department Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, Montana, USA
³Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh, North Carolina, USA
⁴Department of Medicine, University of Southern California, Los Angeles, California, USA
⁵Department of Environmental Medicine, University of Rochester, Rochester, New York, USA
⁶Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, New Jersey, USA
⁷Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland Washington, USA
⁸Department of Nutritional Science and Toxicology, University of California, Berkeley, Berkeley, California, USA
Table of Contents:

Page 3: Supplemental Material, Table S1: Size and zeta potential of TiO2 and ZnO nanoparticles in media

Page 3: Supplemental Material, Table S2: Size and zeta potential of MWCNT in media

Page 4: Supplemental Material, Figure S1: Stability of O-MWCNT, P-MWCNT, and F-MWCNT suspensions in BEGM in the absence or presence of dispersing agents.

Page 5: Supplemental Material, Figure S2: Cytotoxicity in the BEAS-2B Model.

Page 6: Supplemental Material, Figure S3: Cytotoxicity in the RLE-6TN model.

Page 7: Supplemental Material, Figure S4: Individual lab results and summary results for IL-1β release in the THP-1 model exposed to MWCNT variants.

Page 8: Supplemental Material, Hierarchical Model for Reproducibility Analysis
**Supplemental Material, Table S1:** Size and zeta potential of TiO2 and ZnO nanoparticles in tissue culture media (mean ±s.d.)

<table>
<thead>
<tr>
<th>Quality</th>
<th>Technique</th>
<th>P25</th>
<th>Anatase</th>
<th>Nanobelts</th>
<th>ZnO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size in BEGM (nm) (intensity-based)</td>
<td>DLS</td>
<td>374±38</td>
<td>385±85</td>
<td>1765±265</td>
<td>196±13</td>
</tr>
<tr>
<td>Size in F12 (nm) (intensity-based)</td>
<td>DLS</td>
<td>247±7</td>
<td>200±15</td>
<td>1463±39</td>
<td>371±27</td>
</tr>
<tr>
<td>Size in RPMI (nm) (intensity-based)</td>
<td>DLS</td>
<td>204±7</td>
<td>546±11</td>
<td>1590±126</td>
<td>227±9</td>
</tr>
<tr>
<td>Zeta Potential in BEGM (mV)</td>
<td>Zetasizer</td>
<td>-13.6±1.6</td>
<td>-10.9±1.8</td>
<td>-6.7±2.1</td>
<td>-11.0±1.4</td>
</tr>
<tr>
<td>Zeta Potential in F12 (mV)</td>
<td>Zetasizer</td>
<td>-7.7±2.2</td>
<td>-7.9±2.8</td>
<td>-21.5±1.8</td>
<td>-10.8±3.6</td>
</tr>
<tr>
<td>Zeta Potential in RPMI (mV)</td>
<td>Zetasizer</td>
<td>-12.8±0.1</td>
<td>-11.3±0.7</td>
<td>-12.7±4.7</td>
<td>-13.5±0.2</td>
</tr>
</tbody>
</table>

**Supplemental Material, Table S2:** Size and zeta potential of the MWCNT in tissue culture media (mean ±s.d.)

<table>
<thead>
<tr>
<th>Quality</th>
<th>Technique</th>
<th>O-MWCNT</th>
<th>P-MWCNT</th>
<th>F-MWCNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size in BEGM (nm) (intensity-based)</td>
<td>DLS</td>
<td>187±51</td>
<td>247±48</td>
<td>163±13</td>
</tr>
<tr>
<td>Size in RPMI (nm) (intensity-based)</td>
<td>DLS</td>
<td>419±48</td>
<td>375±23</td>
<td>244±4</td>
</tr>
<tr>
<td>Zeta Potential in BEGM (mV)</td>
<td>Zetasizer</td>
<td>-11.8±1.4</td>
<td>-10.5±1.1</td>
<td>-9.9±1.6</td>
</tr>
<tr>
<td>Zeta Potential in RPMI (mV)</td>
<td>Zetasizer</td>
<td>-10.5±0.9</td>
<td>-9.8±1.1</td>
<td>-11.4±1.3</td>
</tr>
</tbody>
</table>
Supplemental Material, Figure S1: Stability of O-MWCNT, P-MWCNT, and F-MWCNT suspensions in BEGM in the absence or presence of dispersing agents [BSA (0.6 mg/mL)±DPPC (0.01 mg/mL)]. (A) The suspension stability index of the MWCNT was determined as a function of time after suspension at 50 µg/mL in BEGM in the absence or presence of BSA, DPPC, or BSA+DPPC. The suspension stability index was calculated as the % of initial MWCNT absorbance (t = 0) at λ=550 nm for time periods of 1, 2, 3, and 24 h. The absorbance measurements were carried out by a UVvis spectrometer (SpectroMax M5e, Molecular Devices Corp., Sunnyvale, CA). (B) The dissolution rate of ZnO in DI H2O, BEGM, and DMEM media. The ZnO dissolution was determined by ICP-MS: 50 µg/mL nanoparticles was suspended in DI H2O, BEGM, and DMEM media at room temperature for 24 h. The suspension was centrifuged at 20,000 g for 1 h, and the zinc concentration in the supernatant was determined by ICP-MS. Data are expressed as means ± SEM; * indicates significance at P < 0.05 compared to the dissolution rate of ZnO in DI H2O.
Supplemental Material, Figure S2: Cytotoxicity in the BEAS-2B Model. A) Percent viable cells relative to no particle control for BEAS-2B Phase I conditions. B) Percent LDH release relative to total lysis (100% cell death) for BEAS-2B Phase I conditions. C) Percent viable cells relative to no particle control for BEAS-2B Phase II conditions. D) Percent LDH release relative to total lysis (100% cell death) for BEAS-2B Phase II conditions. Data are expressed as means ± SEM; * indicates significance at $P < 0.05$ compared to other particles at the same concentration and the “no particle” control.
Supplemental Material, Figure S3: Cytotoxicity in the RLE-6TN model. A) Percent viable cells relative to no particle control for RLE-6TN Phase I conditions. B) Percent LDH release relative to total lysis (100% cell death) for RLE-6TN Phase I conditions. C) Percent viable cells relative to no particle control for RLE-6TN Phase II conditions. D) Percent LDH release relative to total lysis (100% cell death) for RLE-6TN Phase II conditions. Data are expressed as means ± SEM; * indicates significance at $P < 0.05$ compared to other particles at the same concentration and the “no particle” control.
**Supplemental Material, Figure S4:** Individual lab results and summary results for IL-1β release in the THP-1 model exposed to MWCNT variants. Data are expressed as means ± SEM; asterisks indicate significance at *** $P < 0.001$, ** $P < 0.01$, or * $P < 0.05$ compared to F-MWCNT at the same concentration. Daggers indicate significance at ††† $P < 0.001$, †† $P < 0.01$, or † $P < 0.05$ compared to P-MWCNT at the same concentration.
Supplemental Material, Hierarchical Model for Reproducibility Analysis

Within assay, particle and cell line, let $y_{rijk}$ be the normalized response value measured during round $r = 1,2$; for lab $i = 1,...,8$; exposure level $j = 1,...,5$ and replicate $k=1,...,3$. We consider the following two stage hierarchical model:

1) $y_{rijk} = m_{rij} + \varepsilon_{rijk}$, with $\varepsilon_{rijk} \sim N(0,\sigma_r^2)$; (Sampling model)

2) $m_{rij} = \mu_{ij} + e_{rij}$, with $e_{rij} \sim N(0,\tau_r^2)$. (Mean model)

In the foregoing formulation, $m_{rij}$ is the mean response over replicates obtained during round $r$, by lab $i$, for dose $j$. The measurement error $\varepsilon_{rijk}$ is assumed to be Gaussian with mean zero and variance $\sigma_r^2$, assumed to be specific to round $r$. The mean model in (2) assumes a population mean $\mu_{ij}$ that is specific to experimental round $r$ and dose $j$, but aggregates over labs, therefore being interpreted as the overall mean. The error in mean $e_{rij}$ measures deviations of individual lab means $m_{rij}$ from the overall means $\mu_{ij}$ and is assumed to be Gaussian with mean zero and variance $\tau_r^2$.

The model is completed with the following conjugate prior distributions:

1. $\mu_{ij} \sim N(0,\nu_\mu)$,
2. $\sigma_r^2 \sim IG(a_\sigma,b_\sigma)$,
3. $\tau_r^2 \sim IG(a_\tau,b_\tau)$.

Our inference centers on two main quantities of interest, namely: the posterior distribution of the measurement error $\sigma_r^2$, that we interpret as a measure of repeatability in experimental round $r$; and the posterior distribution of the error in mean $\tau_r^2$, which we interpret as a measure of experimental reproducibility in round $r$.

These quantities are estimated with arbitrary precision via Markov chain Monte Carlo simulation. In our analysis we considered diffuse prior information setting $\nu_\mu = 10^8$, $a_\sigma = b_\sigma = a_\tau = b_\tau = 0.1$. Our conclusions are not sensitive to alternative default specification of the prior structure.