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Nanotoxicology: *in Vitro*-*in Vivo* Dosimetry

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Gangwal et al. (2011) addressed an important topic for nanotoxicology about assessing the toxicity of inhaled nanomaterials by recommending relevant concentrations for *in vitro* toxicity testing. Their efforts to select *in vitro* concentrations based on reported occupational exposure levels of inhaled nanomaterials are, indeed, laudable. Their underlying conceptual approach is logical, involving a widely used and well-accepted particle dosimetry model [multiple-path particle dosimetry (MPPD)] to estimate deposited and retained mass doses in the pulmonary alveolar region of nanomaterial-exposed workers. They then expressed these doses as per-unit alveolar surface area in order to select for *in vitro* testing the same alveolar epithelial cell surface area doses. However, while this concept makes good sense when applying it to short-term (daily) deposited doses, it makes less sense and can be highly misleading when the same approach is used for doses that have accumulated in the alveolar region after long-term chronic inhalation exposures of humans. Thus, it is unfortunate that the authors made it a main point to estimate (although crudely and with some questionable assumptions) the dose of inhaled nanomaterials that is retained or accumulated on the pulmonary alveolar surface over a full working lifetime of 45 years of exposure to 1 mg/m³ airborne concentration. Gangwal et al. then converted this 45-year accumulated surface area dose to an equivalent *in vitro* concentration (per square centimeter) as a selection criterion for *in vitro* dosing. Under “Concentrations recommended for *in vitro* testing,” they concluded that the long-term retained human alveolar surface area dose equates to *in vitro* concentrations of 50–68 µg/mL and that

These amounts for a full working lifetime lie within the range of the highest *in vitro* assay concentrations tested in the literature for Ag [silver nanoparticles] and TiO₂ [titanium dioxide nanoparticles] on human, rat, and mouse cell lines. (Gangwal et al. 2011)

These are extraordinarily high concentrations, and unfortunately this article may be viewed as a justification for using such high *in vitro* dosing uncritically. Gangwal et al. (2011) did not discuss anywhere in the article the reasoning behind equating lifetime accumulated doses with doses that are given all at once as a bolus in a short-term *in vitro*

system. The difference in dose rate alone—not considering anything else—spans many orders of magnitude. At best, these extrapolated high *in vitro* concentrations may be labeled as the high-end limit of an *in vitro* study using a wide range of doses.

To their credit, Gangwal et al. (2011) estimated lung surface area doses achieved for a 24-hr exposure to an inhaled concentration of 1 mg/m³ and—as one would expect—extrapolated this to much lower concentrations of 0.17–0.57 µg/mL for equivalent *in vitro* dosing with TiO₂ and Ag nanoparticles. If they had used a more realistic higher value for the human alveolar surface area—as they did for the full working lifetime exposure—the extrapolated equivalent short-term *in vitro* concentrations would have been even lower by about one order of magnitude. Unfortunately, the authors did not emphasize the tremendous differences between actual high doses used in most published *in vitro* studies of nanoparticles and the more realistic much lower *in vitro* doses. For *in vitro* testing, use of a wide range of doses, starting at—or even better—below the 24-hr inhalation equivalent and increasing to a maximum of the lifetime exposure equivalent, could be a practical approach.

With respect to carbon nanotubes (CNTs), Gangwal et al. (2011) reported results only for the full working lifetime exposure scenario and the resulting extrapolated equivalent *in vitro* concentrations. According to the authors, these extrapolated high equivalent *in vitro* concentrations are at the low end of concentrations that have been reported for CNTs in the *in vitro* literature. Implications for selection of realistic *in vitro* exposures to CNTs were not discussed, nor was the more relevant 24-hr exposure scenario for CNTs modeled to derive an equivalent short-term *in vitro* dose. This would have provided a suggested range of *in vitro* dosing for CNTs as pointed out above for Ag and TiO₂ nanoparticles, provided the dosimetry model (MPPD) is applicable for CNTs. Unfortunately, the validity of the MPPD model for fiber-shaped structures of nanosized dimensions was neither explained in sufficient detail by Gangwal et al., nor has it been confirmed and published, specifically for nanofibers and nanotubes. Moreover, a thorough literature search reveals that CNT aerosols at workplaces are not present as individual straight nanofibers, but occur mostly as small and large tangles of different shapes of hollow tubes with unknown effective density (density is not that of solid

carbon) (Han et al. 2008; Methner et al. 2010; Tsai et al. 2009). There is currently no deposition model that could be applied for such nanostructures without additional research to obtain necessary input data.

A careful selection of *in vitro* doses for nanoparticle toxicity testing is very important. Thus, authors, reviewers, and journal editors should be critical when submitting, reviewing, and accepting papers for publication.

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Nanotoxicology: Gangwal et al. Respond

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We appreciate the letter from Oberdörster commenting on the importance of careful selection of *in vitro* doses for nanomaterial (NM) toxicity testing and his assessment of our article (Gangwal et al. 2011). Because the objective of our study was to use limited data on potential human occupational exposure to NMs to identify bounding limits for toxicity testing, we believe our conclusions and Oberdörster’s views to be generally aligned.

Our article described how to apply sparse NM exposure information from manufacturing and R&D (research and development) settings and relevant particle dosimetry model inputs, based on a report of the International Commission on Radiological Protection (1994), to estimate NM mass retained in the alveolar region of the human lung. Modeled alveolar lung surface concentrations (micrograms per square centimeter) were then used to estimate bounding *in vitro* NM solution concentrations (micrograms per milliliter) representative of short-term (24-hr) and long-term (full occupational lifetime of 45 years)

exposure scenarios. In comparing our rough “equivalent” estimates obtained based on lifetime exposure to concentrations currently being used for *in vitro* testing, we indeed intended to highlight that such concentrations represent a high end bounding limit, as Oberdörster has emphasized. Equivalent *in vitro* concentrations based on a 24-hr scenario are intended to represent more realistic short-term exposures.

We agree with Oberdörster that our article (Gangwal et al. 2011) should not be viewed as justification for using very high NM *in vitro* testing concentrations. Rather, we demonstrate the importance of understanding *in vitro* concentrations in the context of the potential for human NM exposure to improve study design and facilitate interpretation of testing results. For NMs currently being tested in the U.S. Environmental Protection Agency’s (EPA) ToxCast project (Dix et al. 2007), we are in fact evaluating multiple concentrations based on consideration of potential exposure and generally have set NM testing concentrations to range from below the 24-hr inhalation exposure equivalent to the full working lifetime equivalent.

As we note in our article (Gangwal et al. 2011) and as Oberdörster has further emphasized, there are significant uncertainties associated with our estimates of exposure and associated dosing concentrations. These include uncertainties associated with screening-level tools available for modeling deposition of engineered nanomaterials and with our understanding of characteristics and properties of materials found in the human environment. In the interest of mining available tools to inform design of toxicity tests for immediate use, we did opt to make significant simplifying assumptions related to particle characteristics and to apply a version of the MPPD model adapted by the developers for application to nanofibers/nanotubes (National Institute for Occupational Safety and Health 2008). The modeled alveolar mass retained for CNTs based on more realistic, short 24-hr inhalation exposure duration is available online (U.S. EPA 2011).

One point that Oberdörster missed in our article (Gangwal et al. 2011) is that we calculated alveolar lung surface concentration using the same low estimate of human alveolar surface area for both the full working lifetime and the 24-hr exposure duration, and thus calculations for both exposure scenarios may be lower by approximately one order of magnitude.

We are pleased that our framework for using available exposure information to inform selection of *in vitro* toxicity testing concentrations is generating important discussion. We believe the issues and limitations raised in our article and by Oberdörster are

important and demonstrate a critical need for continuing research to understand the potential for human exposure to engineered nanomaterials and to design environmentally relevant toxicity testing schemes.

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Bisphenol A in Thermal Paper Receipts: An Opportunity for Evidence-Based Prevention

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The recent report by Taylor et al. (2011) on the pharmacokinetics of bisphenol A (BPA) emphasizes the similarities between humans, monkeys, and mice in the metabolism of this ubiquitous and potentially toxic synthetic chemical. The authors suggested that human exposure to BPA may be “much higher than previously assumed.” They observed that a potentially important nonfood source of exposure to BPA may be the thermal paper used in cash register receipts.

BPA is found in receipt paper (Mendum et al. 2010) and appears to transfer readily from receipts to skin (Biedermann et al. 2010) and to be absorbed transdermally (Zalko et al. 2011). Retail workers, who likely have more frequent exposure to cash receipts containing BPA than other Americans, have been found to have elevated levels of urinary BPA (Lunder et al. 2010). BPA has been

shown to be capable of crossing the placenta (Balakrishnan et al. 2010) and to be toxic during early mammalian development (vom Saal and Hughes 2005). This toxicity is relevant to humans, given the similarities in BPA metabolism observed across species by Taylor et al. (2011). Prenatal exposure of human infants to BPA has been associated with behavioral anomalies (Braun et al. 2009).

There is a sense of déjà vu about this story: In the 1970s polychlorinated biphenyls (PCBs) were widely used in carbonless copy paper (Erickson and Kaley 2011). PCBs were shown to be absorbed through the skin (Carpenter 2006), and prenatal exposures to PCBs were subsequently shown to cause irreversible brain injury to developing fetuses, which resulted in permanent loss of IQ (intelligence quotient) and alterations in behavior (Jacobson and Jacobson 1997). This exposure ended when the manufacture of PCBs was banned in the United States in 1976.

The research of Taylor et al. (2011) contributes to our understanding of the potential harms to the developing fetus from BPA. These findings underscore the need to develop a new U.S. chemical policy that would require toxicological testing of widely used chemicals already on the market and premarket safety testing of all proposed new chemicals (Landrigan and Goldman 2011). The time to presume that chemicals are safe until they are proven beyond all doubt to cause injury to America’s children is past. While research into the effects of exposure to BPA continues, we have an opportunity to act today on the basis of the available evidence to remove BPA from thermal paper, as we strive to protect the health and future intelligence of America’s children.

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