Sparking Connections: Toward Better Linkages Between Research and Human Health Policy—An Example with Multiwalled Carbon Nanotubes

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ABSTRACT

Risk assessment and subsequent risk management of environmental contaminants can benefit from early collaboration among researchers, risk assessors, and risk managers. The benefits of collaboration in research planning are particularly evident in light of (1) increasing calls to expand upon the risk assessment paradigm to include a greater focus on problem formulation and consideration of potential tradeoffs between risk management options, and (2) decreasing research budgets. Strategically connecting research planning to future decision making may be most critical in areas of emerging science for which data are often insufficient to clearly direct targeted research to support future risk assessment and management efforts. This article illustrates an application of the comprehensive environmental assessment approach to inform research planning for future risk assessment and management of one emerging material, multiwalled carbon nanotubes (MWCNTs). High-priority research areas identified for MWCNTs in flame-retardant coatings applied to upholstery textiles included the following: release across the product life cycle; environmental transport, transformation and fate in air, wastewater and sediment; exposure in human occupational and consumer groups; kinetics in the human body; impacts on human health and aquatic populations; and impacts on economic, social, and environmental resources. This article focuses on specific research questions related to human health and how these may connect to future risk assessments and risk management efforts. Such connections will support more effective collaborations across the scientific community and may inform the prioritization of research funding opportunities for emerging materials like MWCNTs.

Key words: comprehensive environmental assessment; engineered nanomaterials; risk assessment; multiwalled carbon nanotubes; research planning

There has been a call upon risk assessors and others to expand upon the risk assessment paradigm by including, for instance, greater focus on problem formulation and consideration of potential tradeoffs between risk management options (NRC, 2009, 2011). Given that these calls come in a climate of increasingly limited budgets, research to support human and environmental risk management decisions must be more efficient and effective. Efficiently and effectively planning research that informs risk assessment and management is a challenge for researchers, risk analysts, policy makers, and others in a variety of fields (Davis and Howden-Chapman, 1996; Lavis et al., 2003).

However, it may be particularly challenging in emerging fields of science, such as engineered nanomaterials (ENMs), in which existing data may be insufficient to clearly direct targeted research to support future assessment and risk management efforts. Indeed, a recent National Research Council (NRC) report notes a clear disconnect between current research and “its relevance to and use in decision making” for ENMs (NRC, 2012). Recent commentaries within the ENM field reinforce the importance of creating stronger ties between the research, risk assessment, and risk management communities (Schug et al., 2013). One commentary evaluating available data on silica nanoparticles notes

that the lack of coherence among the studies renders the picture of potential risk from the materials fuzzy, if not completely opaque (Schrurs and Lison, 2012). Yet, some experts think that the existing data, particularly for specific types of ENM, such as nanoscale silver, should allow policy makers to move forward and make decisions regarding potential human health and environmental risks (Hansen and Baun, 2012).

To facilitate policy decisions on ENM environmental health and safety (EHS), researchers, risk analysts, policy makers, and others need approaches to collaboratively plan, implement, and communicate the outcomes of research that informs risk assessment and management (Davis and Howden-Chapman, 1996; Lavis et al., 2003). Efforts have been made to guide the research community in better connecting laboratory or field research to risk management or policy decisions for EHS (NNI, 2011; NRC, 2012; OECD, 2012b). Nevertheless, a mechanism to consistently and strategically link research, risk assessment, and risk management would benefit not only those working to understand, use, and manage ENMs but also those working with other emerging materials, chemicals, or technologies.

One such mechanism is comprehensive environmental assessment (CEA): an approach used by the U.S. Environmental Protection Agency (EPA) to identify priority research gaps and risk-related tradeoffs for informing decisions that protect human health and the environment (Davis, 2013; Powers et al., 2012). The CEA approach is discussed in detail elsewhere but includes certain core principles: engaging diverse perspectives, focusing on prioritization, and linking research to future assessment and decision making (Davis, 2013; ICF International, 2011; Painter et al., 2014; Powers et al., 2012, 2014; RTI International, 2012; U.S. EPA, 2013a, 2010a, 2010b, 2012c). The focus of this article is on outcomes of a recent application of CEA to identify research priorities for multiwalled carbon nanotubes (MWCNTs) in flame-retardant coatings applied to upholstery textiles. More information on the rationale for selecting MWCNTs in flame-retardant coatings applied to upholstery textiles and on studies identified as potentially relevant to the use of MWCNTs in this application is available in U.S. EPA (2013a). Compared with other efforts to identify research priorities for ENMs (e.g., NNI, 2011; NRC, 2012; OECD, 2012b), the outcomes of this CEA application have two key distinguishing characteristics. First, experts engaged in the CEA process prioritized research gaps based on the robustness of available data and importance specifically for future risk assessments. This differs from other lists of ENM research gaps, which neither identify particular research needs as priorities nor compare the urgency of specific research needs either within or across different domains (e.g., human health effects compared with life cycle research). Second, experts participating in the CEA process developed specific research questions for the research gaps identified as most pressing. This contrasts with other lists of ENM research priorities, which provide only general research topics of interest (e.g., human exposure data). As a result, the outcomes of the MWCNT CEA may facilitate the translation of these identified research gaps into support for research efforts targeted to meet the needs of future risk analyses. Specifically, collaborative efforts by individuals and organizations across the scientific community to plan and implement the research prioritized through the CEA process can produce data that experts have identified as important for future MWCNT risk assessment and management efforts. In the CEA process applied to MWCNTs, a group of 13 expert stakeholders (hereafter, “CEA participants”) representing a cross-section of sector (e.g., academia, industry, government) and technical (e.g., toxicology, materials science, ecology) perspectives reviewed a

FIG. 1. Comprehensive environmental assessment (CEA) importance/confidence matrix [adapted from RTI International (2012)]. Areas identified as “research priorities” are those that experts most frequently rated as (1) highly important to consider in future risk assessment and (2) not confident in available data to support risk management decisions (top right, red box of matrix). Lower priority research areas are denoted as orange, yellow, or green, in descending order of priority.

draft case study of MWCNTs in flame-retardant coatings applied to upholstery textiles (U.S. EPA, 2012b) and participated in a face-to-face workshop to finalize a set of research priorities. Potential research priorities discussed at the workshop were identified through a series of rating exercises previously completed by a larger group of experts using a software tool remotely (Powers et al., 2014; RTI International, 2012). After a structured discussion of the potential priorities identified through the remote rating process, the CEA participants attending the workshop used the software tool a final time to individually rate research areas based on (1) importance to support future risk assessments of MWCNTs and (2) confidence in the adequacy of available data to support a risk management decision. Areas that participants most commonly rated as (1) of high importance to risk assessment and (2) of low confidence with regard to the adequacy of the available data to support decision making were identified as high research priorities (Fig. 1).

High-priority areas for research on MWCNTs in flame-retardant coatings applied to upholstery textiles included the following: release across the product life cycle; environmental transport, transformation and fate in air, wastewater and sediment; exposure in human occupational and consumer groups; kinetics in the human body; impacts on human health and aquatic populations; and impacts on economic, social, and environmental resources (RTI International, 2012; U.S. EPA, 2013a) (Fig. 2). Thus, the identified priority research areas span multiple disciplines, and efforts by the scientific community to address these data gaps could inform several types of analyses (e.g., life cycle assessments, environmental risk assessments, human health risk assessments). This article focuses on the subset of priority research areas that relate to human health and the ways in which researchers might investigate these areas to inform future decision making for MWCNTs within this domain. All of the high-priority areas and the process by which they were identified are discussed in RTI International (2012) and U.S. EPA (2013a).

The objective of this Forum article is to provide information regarding priority data gaps to individuals and organizations in the scientific community who are interested in planning research to collect data that could be used in future human health risk assessment and management efforts for MWCNTs, particularly MWCNTs used in flame retardant textiles. To accomplish its objective, this article discusses specific research questions relevant to human health risk assessment. The subset of data gaps identified by CEA participants and discussed here relates to human health impacts, human inhalation exposure in occupational and consumer groups, and kinetics in the human body. Data gaps in each area are discussed within the context of available data, guidance, and specific experimental design considerations.

SUPPORTING FUTURE ASSESSMENT AND RISK MANAGEMENT OF MULTIWALLED CARBON NANOTUBES: RESEARCH PRIORITIES RELATED TO HUMAN HEALTH

When considering options for MWCNT risk management related to protecting human health, the CEA participants noted that human health risk can be mitigated or avoided by establishing acceptable exposure limits for workers and members of the general population. To support the development of acceptable MWCNT exposure limits, decision makers in regulatory agencies and industry are actively evaluating the potential health impacts of these materials in specific human populations (i.e., workers, consumers, susceptible individuals). This type of information is generally compiled and utilized within the context of a human health risk assessment (HHRA). Although understanding the health risk posed by human exposure to MWCNTs is critical for the decision-making process, additional information on the economic and possibly social impacts of manufacturing and using MWCNTs would also need to be considered. Thus, risk managers would likely use information from a cost-benefit analysis, or other economic assessment, in addition to an HHRA. Nevertheless, the focus of this article will be on identifying information to support an HHRA, with details related to cost-benefit analysis left for future publications [see U.S. EPA (2013b) for available guidance on preparing economic analyses].

U.S. EPA HRAs generally follow the paradigm established by the National Academy of Sciences (NRC, 2009; U.S. EPA, 2012a). This paradigm describes a group of interconnected processes for performing an HHRA that include hazard identification, dose-response assessment, exposure assessment, and risk characterization. In hazard identification, available data on biological endpoints are used to determine if a material is likely to pose a hazard to human health, and, if so, what type of hazard it may pose. In dose-response assessment, data are used to estimate the amount of exposure that may produce a health effect in humans. The exposure assessment portion of the HHRA process seeks to provide an estimate of exposure to a material for a specific population within a specific context. In risk characterization, the results of an exposure assessment are integrated with the results of a dose-response assessment to provide characterizations of human risk, which include identification of susceptible populations and descriptions of uncertainty.

The CEA participants identified the potential for MWCNTs in flame-retardant coatings applied to upholstery textiles to cause non-cancer health effects in humans as a priority area of investigation needed to support HHRA. They also considered cancer to be an important health outcome to consider for future

MWCNT risk assessments. However, because the CEA participants had more confidence in the ability of currently available data on the carcinogenic potential of MWCNTs to support HHRA, research on non-cancer health effects was determined to be a higher priority for future resource allocation (Figs. 1–2; Reprinted with adaptation from Science of the Total Environment, Vol 470–471, Powers, CM, Grieger, KD, Hendren, CO, Meacham, CA, Gurevich, G, Lassiter, MG, Money, ES, Lloyd, JM, and Beaulieu, SM, A web-based tool to engage stakeholders in informing research planning for future decisions on emerging materials, pp. 660–668, Copyright (2014), with permission from Elsevier) (RTI International, 2012; U.S. EPA, 2013a). A variety of exposure scenarios can be envisioned for MWCNTs in upholstery applications, each of which might lead to non-cancer health effects. The CEA participants considered research on inhalation exposures in occupational and consumer settings to be especially important to support HHRA. They also thought that information is urgently needed to better understand the kinetics of MWCNTs in the human body, especially absorption, metabolism, and excretion, particularly following inhalation exposure. These topics are discussed below in the context of carrying out hazard identification, dose-response evaluation, and exposure assessment within an HHRA.

Hazard Identification and Dose-Response Assessment of Human Non-Cancer Health Effects of MWCNTs

Information for hazard identification and dose-response assessment often comes from studies of laboratory animals, particularly for substances that are new to the marketplace. As shown in Table 1, the CEA participants suggested that researchers conduct acute and chronic rodent bioassay studies to support hazard identification and dose-response assessment for the non-cancer health effects of MWCNTs, which were identified as a research priority (Fig. 2). As discussed below and indicated in Figure 2, they determined that inhalation is the route of exposure of primary concern. Thus, for the studies described in Table 1, participants suggested exposing rodents via inhalation. In addition, CEA participants recommended that researchers collect data on how physicochemical characteristics influence health effects observed in rodent inhalation studies. In the interest of public health protection, the CEA participants also suggested that additional rodent studies be conducted to evaluate dose response in models of susceptible populations, including children and the elderly. Due to differences in physiological parameters, these populations may be more vulnerable to adverse health outcomes. For such cases, literature suggests that study designs involving early-life exposures and late-life sacrifice can markedly increase rodent bioassay sensitivity (Maronpot et al., 2004). Based on initial evidence with MWCNTs, immune-compromised individuals may also be particularly susceptible to MWCNT non-cancer effects (Ryman-Rasmussen et al., 2009b).

As indicated in Table 1, of three types of experiments that CEA participants recommended to support hazard identification and dose-response, the authors suggest prioritizing research on the relationship between the physicochemical properties of MWCNTs and health effects following inhalation exposures in rodents. The rationale for prioritizing this research relative to the other questions CEA participants identified in this area rests on the importance of understanding these relationships in designing follow-up studies using rodent models of chronic exposure or susceptible populations (e.g., immune-compromised individuals). Moreover, initial studies to elucidate relationships between physicochemical properties of MWCNTs and health effects could be carried out using *in vitro* models to expedite the

TABLE 1. Priority Research Questions to Support Human Health Risk Assessment as Identified by CEA Participants [Adapted from RTI International (2012)]

Studies to support identification and dose-response assessment of human non-cancer health effects							
Suggested priority (Authors) ^a	Risk management	Informing management	Assessment	Informing assessment	Research	Estimated finances (\$) ^b	Estimated time frame
1	Regulation to promote the production or use of MWCNTs with reduced toxicity	Safe exposure limits for a variety of MWCNTs with different physicochemical properties	HHRAs for a variety of MWCNTs with different physicochemical properties	Toxicity in rodents as a function of particle characteristics	Perform experiments to test impacts of particle characteristics on toxicity in rodents exposed by inhalation	\$2 M for 2 year rodent bioassay including an additional year for setup and analysis	3 years
2	Implement appropriate control technology; or, if available technologies are inadequate to protect human health, consider a moratorium on MWCNT production and use	Safe exposure limits for consumers or workers	HHRA for the relevant material to be used in consumer or occupational exposure scenarios	Rodent toxicity data with exposure relevant material	Conduct acute and chronic rodent bioassay studies after inhalation exposure at relevant doses using well-characterized material	\$2 M for 2 year rodent bioassay including an additional year for setup and analysis	3 years
3	Implement appropriate control technology; or, if available technologies are inadequate to protect human health, consider a moratorium on MWCNT production and use	Safe exposure limits for susceptible individuals	HHRA for the relevant material to be used in consumer or occupational exposure scenarios for susceptible populations	Rodent toxicity data with exposure relevant material	Perform experiments to test impacts of exposure on rodent models of susceptible populations (e.g., immune-compromised individuals)	\$1 M	3 years
Studies to support occupational exposure assessment							
1	Engineering controls and personal protective equipment	Safe industrial exposure limits for workers	HHRA for the relevant material to be used in occupational exposure scenarios	Occupational exposure at current MWCNT processing facilities	Evaluate exposure to workers at manufacturing facilities to identify key steps in exposure and to assess the effectiveness of engineering controls and personal protective equipment	\$100 k	1 year
2	Engineering controls and personal protective equipment	Safe industrial exposure limits for workers	HHRA for the relevant material to be used in occupational exposure scenarios	Impact of co-factors (e.g., solvents, resins) on inhalation exposure	Analytical and rodent studies to examine effect of co-factors on particle size and behavior in the lungs (i.e., deposition, translocation, and removal)	\$2 M for 2 year rodent bioassay including an additional year for setup and analysis	3 years
Studies to support consumer exposure assessment							
1	Regulation of acceptable matrices for use with MWCNT flame retardants	Concentrations of MWCNTs in indoor air as a function of matrix material	HHRA for the relevant material to be used in consumer exposure scenarios, including those for sensitive populations (e.g., children)	Effect of matrix on particle release	Accelerated weathering tests to determine how particle functionalization and matrix affect aging and release to air, including quantification (i.e., the number of MWCNTs released, concentration in air) and characterization (e.g., size distribution)	\$300 k	2–3 years

TABLE 1. Continued

Studies to support identification and dose-response assessment of human non-cancer health effects							
Suggested priority (Authors) ^a	Risk management	Informing management	Assessment	Informing assessment	Research	Estimated finances (\$) ^b	Estimated time frame
Kinetic studies to support HHRA							
1	Regulation to promote the production or use of MWCNTs with properties that minimize their absorption potential	Safe exposure limits for a variety of MWCNTs with different physicochemical properties	HHRAs for a variety of MWCNTs with different physicochemical properties	Absorption potency as a function of particle characteristics for three representative routes of exposure: oral, inhalation and dermal	Determine particle properties that influence extent and rate of absorption across mammalian lung epithelial tissue, GI luminal epithelia, and dermal layers	\$5 M	5 years
2	Regulation to promote the production or use of MWCNTs with properties that maximize their potential for clearance	Safe exposure limits for a variety of MWCNTs with different physicochemical properties	HHRAs for a variety of MWCNTs with different physicochemical properties	Half-life as a function of particle characteristics	Determine particle properties that influence clearance of MWCNTs in rodents, including degradation of particles within cells and/or tissues	\$800 k (\$300 k for initial analytical technique development [e.g., tracer methods] and \$500 k for rodent studies)	5 years (2 for initial analytical technique development and 3 for rodent studies)
3	Implement appropriate control technology; or, if available technologies are inadequate to protect human health, consider a moratorium on MWCNT production and use	Safe exposure limits for consumers or workers	HHRA for the relevant material to be used in consumer or occupational exposure scenarios	Identification of metabolic pathways for MWCNTs	Rodent studies to detect and measure MWCNT metabolites in tissues following inhalation exposure to the relevant material	\$775 k (\$275 k for initial analytical technique development and \$500 k for rodent studies)	4 years (2 for initial analytical technique development and 2 for rodent studies)
						\$500 k (rodent studies)	

^aPriorities are based on currently available science as well as input from CEA participants presented in RTI International (2012).

^b"Estimated finances" were determined by the CEA participants based on their experience with planning and conducting scientific research. These estimates are intended to provide a general indication of the level of support researchers might request from available funding sources.

identification of MWCNT types that would benefit from further evaluation in subsequent chronic studies in rodents (Hartung and Sabbioni, 2011; Nel et al., 2013; Wang et al., 2013). Finally, studies on how different physicochemical properties (e.g., AlO₃ functional groups vs. COOH functional groups) influence biological response in *in vitro* or *in vivo* models may provide insight into the mechanism(s) through which MWCNT exposures lead to health effects (Nel et al., 2013). Mechanistic data could subsequently inform the development of adverse outcome pathways as a way to organize data linking biological response at the molecular level to a biological level relevant to risk assessment (e.g., individual or population response) (Ankley et al., 2010; OECD, 2013). Currently available information related to MWCNT non-cancer health effects, physicochemical properties, and potential approaches to build on existing work are discussed below. To date, the non-cancer health effect that researchers have most commonly observed following MWCNT inhalation is pulmonary inflammation (Pauluhn, 2010). Altered immunological function has also been observed following acute inhalation exposure to MWCNTs (Mitchell et al., 2009). As noted above, the CEA participants emphasized the need to characterize MWCNT physicochemical properties in future studies on these and other potential non-cancer health effects that may result from inhalation exposure. A recent review characterizes the relationship be-

tween some physicochemical characteristics and pulmonary effects following inhalation exposures in rodents, but notably no chronic studies (>90 days) were available to include in the analysis (Gernand and Casman, 2014).

Research on the influence of physicochemical properties on subsequent health effects is difficult due to a general lack of information in the literature on MWCNT formulations in products on the market. Based on the literature that is available, physicochemical characteristics of MWCNTs in flame-retardant coatings applied to upholstery textiles will likely vary but include relatively: (1) short lengths [e.g., 1–2- μ m MWCNTs are more flame-retardant than those 0.5–40 μ m (Pack et al., 2009)]; (2) high aspect ratios [e.g., aspect ratio of 150 demonstrated better flame-retardant performance than one of 49 (Cipiriano et al., 2007)]; and (3) acidic surface coatings or functional groups [e.g., carboxylic acids, phenols, carbonyls (Goncalves et al., 2012)]. Although the lengths of MWCNTs may be in the μ m range, these materials are considered ENMs under the National Nanotechnology Initiative (NNI) definition (i.e., materials that are intentionally developed with at least one external dimension between ~1–100 nm and exhibit unique properties resulting from size) (NNI, 2011). The overlap of the two ranges in length reflects the data available from the referenced study; more data on physicochemical properties of MWCNTs in flame-retardant applications may help to

clarify how tube length influences the flame-retardant behavior of the materials. In addition, examples from the literature suggest that MWCNTs would likely be used in combination with conventional fire retardants or other materials to pass flammability tests in most applications, including upholstery textiles (Morgan, 2006). The addition of these other flame-retardant materials could influence MWCNT physicochemical characteristics and subsequent health effects. More discussion on data identified as relevant to MWCNT physicochemical characteristics in flame-retardant textile coatings is available in U.S. EPA (2013a).

Understanding the physicochemical properties of MWCNTs that go into products is just one component of hazard identification for these materials. Hazard identification studies on MWCNTs incorporated into products, like flame-retardant coatings applied to upholstery textiles, are also important. However, very little information is currently available on the behavior and potential effects of ENMs, including MWCNTs, after they have been incorporated into products and/or undergone the variety of transformation processes ENM products are likely to encounter in the environment (e.g., photo-oxidation, weathering, agglomeration) (Nowack et al., 2012). Given the lack of information on MWCNT product formulations and transformation products, the CEA participants offered a “broad net” research approach for identifying potential health concerns. They concluded that the needs of HHRA would be best served by research using the rodent bioassays suggested in Table 1 for several types of MWCNTs, including the following: MWCNTs as produced by manufacturers (i.e., including any added surface coatings or functional groups); MWCNTs in master batches [i.e., including other substances necessary for a particular application (e.g., reducing flammability)]; MWCNTs embedded in a textile matrix; and MWCNTs in a textile matrix after exposure to UV light or washing (e.g., see Wohlleben et al. (2013) for methods). Notably, each of these materials would need to be characterized to the extent that results could be compared across studies from different laboratories.

Although no consensus yet exists on which material properties are essential to characterize for these types of comparisons, a variety of resources are available (ISO, 2012). It may be that researchers could most efficiently develop a data set to inform HHRA by collaborating with risk assessors and end product manufacturers to agree upon a set of materials and parameters to characterize ahead of time. This type of approach could reduce the time and budgetary resources required to carry out chronic rodent studies for multiple, well-characterized materials. The National Institute of Environmental Health Sciences is currently implementing this type of collaborative effort to develop research using an agreed-upon set of laboratory-produced ENMs (Schug et al., 2013).

To conduct these types of collaborative research efforts, researchers may refer to guidance from the Organisation for Economic Co-Operation and Development (OECD) and other organizations on the design of toxicity studies to support HHRA (OECD, 2009a). Researchers investigating the health effects of MWCNTs may consult these guidelines when selecting certain study design elements that commonly impact the utility of data for dose-response assessment (e.g., the number of animals per dose group, dose range, the number of dose levels tested). However, adaptations to the standard guidelines are necessary when they are applied to the testing of ENMs, especially for studies using the inhalation route (OECD, 2009). For example, specific guidance is available for ENM sample preparation and dosimetry for safety testing (OECD, 2012a). Also, the OECD test guidelines for acute and subacute inhalation toxicity include only very lim-

ited histological examination at autopsy. In studies investigating the toxicity of ENMs by inhalation, detailed histological examination of the entire respiratory tract may be more appropriate. Furthermore, researchers may also wish to consider additional endpoints (i.e., extrapulmonary effects, including immunotoxicity) and longer exposure (i.e., >90 days), or post-exposure observation periods (e.g., 3–6 months) to more fully characterize potential toxicity.

As noted above, the selection of MWCNTs to include in *in vivo* studies could be informed by *in vitro* studies that evaluate the relationship between physicochemical characteristics of MWCNTs and biological response (Wang et al., 2013). Researchers might build off of efforts with multiple types of ENMs, including MWCNTs, that used a suite of mechanism-based *in vitro* assays for high-throughput screening of MWCNTs with distinct physicochemical characteristics (Nel et al., 2013). Results of high-throughput *in vitro* screens could then be validated with a relatively small number of *in vivo* studies (Nel et al., 2013). Notably, assay validation is an important step for any *in vitro* screening approach, and several factors that are unique to ENMs would be important to consider in developing assays for MWCNT toxicity testing (e.g., agglomeration, surface reactivity, readout interference, artifacts) (Hartung and Sabbioni, 2011; Nel et al., 2013; Wang et al., 2013). For a review of strengths and limitations of *in vivo* and *in vitro* approaches to ENM toxicity testing see Hartung and Sabbioni (2011).

Related to considerations about experimental design for *in vitro* or *in vivo* studies is the availability of analytical techniques adequate for identifying, quantifying, and characterizing MWCNTs in environmental and/or biological samples. Although some analytical techniques are available, many cannot accurately characterize MWCNTs in complex matrices (von der Kammer et al., 2012). Better methods are needed to extract, clean up, separate, and store MWCNTs to improve efficiency, sensitivity, and specificity. Thus, the CEA participants suggested that one research priority is to develop improved analytical techniques. The availability of these improved methods would facilitate all research in this field and would build on an ongoing collaboration to develop methods for evaluating MWCNT release from polymer matrices (ILSI, 2013). More discussion on experimental design considerations and analytical methods for MWCNTs is available in U.S. EPA (2013a).

MWCNT Exposure Assessment in Occupational and Consumer Settings

The CEA participants identified information on occupational and consumer exposures to MWCNTs as particularly important to support HHRA (Fig. 2). In both contexts, exposure may occur by ingestion, inhalation, or dermal contact. But, as discussed below and indicated in Figure 2, CEA participants considered inhalation exposure to be the primary exposure route of concern because of limited absorption of MWCNTs by the oral and dermal routes, particularly with respect to their use in flame retardant materials.

Based on the CEA participants' discussions of occupational exposures, potential risks could arise due to inhalation of MWCNTs during any part of the manufacturing process. Many facilities use engineering controls and require workers to wear personal protective equipment to minimize exposure to MWCNTs. However, the level of worker protection varies across facilities; and, in some cases, the filtration technologies and personal protective equipment used may not be appropriate for or sufficiently protective against exposures to nanomaterials (Dahm et al., 2012). Therefore, occupational exposure might still occur.

FIG. 2. Schematic representation depicting expert stakeholder prioritization of areas in the CEA detailed framework based on the CEA importance/confidence matrix (Fig. 1) and available information on multiwalled carbon nanotubes (MWCNTs) [adapted from RTI International (2012)]. Final MWCNT research priorities are shown as blue boxes to denote that the majority of participants selected the area as “important to consider in a future risk assessment of MWCNTs”; sub-areas of the research priorities are denoted with colors corresponding to the matrix (Fig. 1) (i.e., red denotes “research priorities” whereas areas in orange, yellow, and green are, respectively, lower priorities). Areas in white denote those most frequently rated of lower importance to consider in future MWCNT risk assessments. More information on the research priorities and the process for identifying them is available in RTI International (2012) and U.S. EPA (2013a).

The CEA participants suggested that if workers cannot be adequately protected from exposure through the use of engineering controls and/or personal protective equipment, it may be necessary to pursue alternative approaches. To inform risk management options, the CEA participants suggested that research be done to evaluate exposure to workers at current MWCNT processing facilities to assess the effectiveness of engineering controls and personal protective equipment (Table 1). As with research to support hazard identification and dose-response assessment, discussed above, it is also important for exposure re-

search to include careful characterization of the specific test material because the physicochemical properties of the material influence the potential for exposure. Because MWCNTs undergo physical and chemical processing before they are incorporated into flame-retardant applications, some exposure considerations are specific to the occupational exposure setting. After synthesis, MWCNTs are typically purified using physical (e.g., flocculation, microfiltration, centrifugation) or chemical (e.g., acid treatment) techniques (Hou et al., 2008). In addition, before application to products, MWCNTs generally require sur-

face functionalization and dispersal in water or organic solvents (Saeed, 2010). These processes can dramatically alter the physicochemical properties of the material. The CEA participants recommended that research be conducted (1) to evaluate exposure to workers before and after each step of the manufacturing process, and (2) to explicitly assess the impacts of co-factors used in manufacturing processes (e.g., solvents and resins) on the deposition, translocation, and subsequent removal of MWCNTs from the lungs (Table 1).

Of these two research areas, authors recommend prioritizing data on worker exposure levels to MWCNTs over investigations on the impact of co-factors used in manufacturing processes (Table 1). Some information on worker exposure levels to MWCNTs is already available; thus, additional research in this area may require a lower level of investment to inform the selection of risk management options specific to MWCNTs. Although ideally, funds would be available for parallel investigations on the influence of MWCNT co-factors, if necessary, these studies could be pursued subsequent to occupational exposure evaluations. In the interim, it may be beneficial to pursue *in vitro* studies to identify which co-factors would be most informative to evaluate through follow-up *in vivo* experiments.

Collecting new data on potential worker exposure levels will require multiple instruments and analytical techniques (Lehman et al., 2011). While a number of techniques to detect, measure, and characterize MWCNTs are available, each has limitations related to detection limit and/or the range of morphological measurements available. For instance, researchers can use the National Institute for Occupational Safety and Health (NIOSH) Manual of Analytical Methods (NMAM) 5040 to estimate MWCNT mass concentrations in occupational air; but, this method measures elemental carbon as a surrogate for MWCNTs and does not automatically account for background contributions or other forms of particulate carbon (Birch, 2003; NIOSH, 2010). Additional information on techniques to detect, measure, and analyze MWCNTs is available in U.S. EPA (2013a). To help address challenges associated with evaluating occupational exposures to ENMs, including MWCNTs, the NIOSH Nanotechnology Research Center is currently working (1) to produce a series of guidance documents, (2) to develop sampling techniques and analytical methods, and (3) to carry out scientific investigations to inform risk management in occupational facilities (NIOSH, 2012). Thus, researchers interested in evaluating potential occupational exposures to MWCNTs may consider collaborating with NIOSH to maximize resources devoted to this research area.

According to the CEA participants, consumer exposure depends on MWCNT release rate from upholstered products. The long lifespan of upholstery textiles (>10 years) suggests that releases impacting consumer exposure could occur over several years. Different MWCNT flame-retardant production processes are expected to result in differences in release rates and release forms from the textile matrix. These parameters, in turn, will affect the magnitude of exposure during consumer use and the form of the material to which consumers are exposed (Motzkus et al., 2012). For example, regular use of upholstered furniture could abrade the textile surface and release small amounts of free or matrix-bound MWCNTs into the air. The integrity of a flame-retardant coating depends on the strength of the formulation that bonds it to the textile surface (Som et al., 2011), and the release rate of MWCNTs will depend, to some extent, on the rate of polymer matrix degradation (Köhler et al., 2008). Thus, the CEA participants noted that regulations pertaining to the type of matrix used with MWCNT flame retardants might help to avoid or mitigate potential risks from consumer inhala-

tion exposures (Table 1). To inform such regulations, research is needed to determine how the composition of a polymer matrix affects the potential release rate of MWCNTs to indoor air. Although data from plastic polymers (polymethyl methacrylate and polyamide-6) suggest that the composition of the matrix influences the morphology of ENMs (e.g., MWCNTs) released from the polymer, no data have been identified examining this parameter for textiles (Motzkus et al., 2012). Again, as noted above for research related to human health effects and occupational exposure, it is essential that researchers fully characterize the composition of the material released in accelerated weathering tests as the specific physicochemical properties of the material will influence consumer exposure.

Understanding Kinetic Properties of MWCNTs

Kinetic properties are key determinants of exposure and hazard. Specifically, kinetic properties describe a material's absorption, distribution, metabolism, and excretion in an organism. Quantification of these properties is useful for the development of physiologically based pharmacokinetic (PBPK) models to describe the cellular and systemic behavior of MWCNTs following exposure by various routes. Accordingly, the CEA participants identified kinetic properties, especially absorption, metabolism, and excretion, as research topics important to support HHRA (Fig. 2). When considering research priorities related to human health effects and occupational and consumer exposures, the CEA participants highlighted the importance of exposures by inhalation based on preliminary evidence suggesting limited absorption of MWCNTs by the oral and dermal routes. However, given the limited data available, research on kinetics is needed for all routes of exposure: oral, inhalation, and dermal.

To date, research on MWCNT absorption has focused on the inhalation route. In general, inhaled MWCNTs appear to be biopersistent (reviewed in Aschberger et al., 2010). Based on studies in rodents, following intratracheal instillation or inhalation exposure, MWCNTs can remain in the lung for an extended period, up to 6 months (Deng et al., 2007; Elgrabli et al., 2008; Ma-Hock et al., 2009; Pauluhn, 2010). In mice, translocation of MWCNTs to the pleura and subpleura has been demonstrated (Porter et al., 2010; Ryman-Rasmussen et al., 2009a). As discussed below, given the expected persistence of MWCNTs in the lung after inhalation exposure, CEA participants recommended evaluating which physicochemical properties of MWCNTs (e.g., length, surface coating) influence clearance (Table 1).

Only one study has thus far evaluated systemic distribution and excretion of MWCNTs following oral, inhalation, or dermal exposure. After 28 days of oral exposure, most MWCNTs administered to mice were found within the feces, stomach, and intestines with no detectable transport into the blood or obvious metabolism (Deng et al., 2007). No studies were identified that evaluated MWCNT metabolism.

As is true for health effects and exposure, the kinetics of MWCNTs are expected to vary according to their specific physicochemical properties (Jain et al., 2011). To mitigate or avoid potential risks related to MWCNT exposure in humans, the CEA participants noted that risk managers might consider regulations limiting MWCNT production or use to those with properties that minimize their absorption potential or maximize their potential for clearance (Table 1). To inform such regulations, research is needed to determine the particle properties that influence (1) the extent and rate of absorption across mammalian lung epithelial tissue, GI luminal epithelia, and dermal layers, (2) degradation of particles within a cell or tissue, and (3) the potential for clearance, especially from the lungs. Once these

properties are identified, it may be possible to design MWCNTs with properties that decrease their bioavailability and persistence while maintaining their efficacy as flame retardants. Research to link MWCNT particle properties to target cell and subcellular disposition and distribution will also support future PBPK modeling efforts.

The CEA participants identified MWCNT metabolism as another potential source of risk because of the possibility that metabolites may be more toxic than the original exposure material. Even though there is some question regarding the ability of MWCNTs to be absorbed, this does not eliminate the potential for metabolism to play an important role in toxicity. As mentioned above, inhaled MWCNTs persist in the lung, where they, or more likely their surface coatings, may be subject to metabolism. Moreover, depending on the physicochemical properties of the specific exposure material, its metabolites may be absorbed even if the MWCNT itself is not. Thus, the CEA participants recommended that the metabolic potential of MWCNTs be investigated in rodents exposed by inhalation (Table 1).

Ideally, evaluations of MWCNT absorption, metabolism, and clearance would take place in parallel, although investigations specific to each exposure route would take place separately. If resources do not allow parallel investigation efforts, then authors suggest a focus on absorption first, followed by clearance, and subsequently metabolism (Table 1). The rationale for this prioritization rests on the understanding that clear evidence of absorption across mammalian epithelial barriers could inform funding levels for follow-up work on clearance or metabolism.

SUMMARY

In the application of CEA described here, a group of experts with diverse perspectives identified research priorities for MWCNTs in a particular application. Perhaps most importantly, the CEA participants connected many of these priorities to future risk assessment and risk management efforts to protect human health. These linkages provide a critical foundation for individuals and organizations across the scientific community to strategically plan research such that resources are maximized to inform decision making (NRC, 2009).

Notably, the availability of data for future ENM risk assessments will depend not only on scientific advances in methods to detect, characterize, and understand ENMs but also on factors unrelated to science, such as budget constraints (NRC, 2009, 2012). Efforts to conduct the research described above will benefit from the support of appropriate funding sources, including public, private, and international initiatives. An evaluation by the NRC suggests “strategic realignment” of federal dollars devoted to ENM EHS research into priority research areas identified by NRC and the NNI to best support efforts to close the gap between available data and the development of “safe” ENMs (NRC, 2012). The priorities identified in the CEA Workshop and reflected in their research suggestions (Table 1) generally align with those put forward by NRC and NNI. For instance, the rodent bioassays that CEA participants suggest for evaluating potential non-cancer effects of MWCNTs could fall within the NRC priority research category of “common processes affecting both potential hazard and exposure”. Similarly, the analytical and rodent studies the CEA participants identified for informing risk management decisions about occupational exposure would fall under the NNI’s research need “understand processes and factors that determine exposures to nanomaterials”. The CEA approach complements and expands upon these previous efforts

to identify research priorities through its focus on one specific type of ENM (i.e., MWCNTs) in a particular application, which generally allowed for the development of more specific research questions for the identified priority areas. This specificity may support the implementation of these research goals in the scientific community. Similarly, the focus on prioritization may inform decision makers as they evaluate how to allocate research resources in the near-term (e.g., pursuing areas described above) versus longer-term (e.g., pursuing more detailed information in other areas identified as important, such as carcinogenicity).

Given the critical relationship between funding and implementing research priorities, the CEA participants provided estimates of the funding levels for each particular research question (Table 1). These estimates rely on the experience of the CEA participants with planning and conducting scientific research and are intended to provide a general indication of the level of support researchers might request from available funding sources. Also included in Table 1 are the CEA participants’ estimates of the time needed to complete the identified research. Based on this information, completing the research discussed in this article will likely take a minimum of 3–5 years. Together, this information on projected time and cost can inform the efforts of funding agencies and research managers to strategically prioritize specific research in order to move the field forward such that risk assessors will have the information they need to adequately inform policy makers.

Developing policy decisions from the resulting risk assessments will take additional time, particularly because risk managers may incorporate information on a wide variety of other parameters. Indeed, risk managers developing plans to mitigate or avoid ENM risk may consider not only the results of risk assessments based on available data but also (1) comparative assessments or ranking of relative risk between different ENMs with unique formulations, applications, or uses, (2) potential risk across the product life cycle, and (3) ethical, legal, and societal implications (e.g., public understanding of risks and benefits, economic benefits of ENMs) (NNI, 2011). Thus, the development of risk management plans for ENMs will likely involve the evaluation of potential tradeoffs between using, for instance, one type of ENM versus another (e.g., MWCNTs or carbon nanofibers), one ENM type with different formulations (e.g., carboxylated-MWCNTs or NH_3^+ -MWCNTs), or ENMs versus non-nanoscale materials (e.g., MWCNTs or traditional flame-retardants). The CEA participants recognized this likelihood and incorporated the comparison of multiple types of MWCNTs into many of the specific research questions they developed (Table 1). The pursuit of these research questions is expected to provide critical support to future risk assessments that will subsequently inform risk managers in developing plans to mitigate or avoid ENM risks while accounting for potential tradeoffs within any one plan.

Ultimately, pursuing the research described above, as well as the other priorities that CEA participants identified and key research objectives identified elsewhere (NNI, 2011; NRC, 2012; OECD, 2012b), will require close collaboration throughout the scientific community. For instance, decision makers responsible for developing funding opportunities [e.g., National Science Foundation Science to Achieve Results (STAR) grants and National Institutes of Health Requests for Proposals] could utilize the research priorities that CEA participants identified to plan separate, but related, funding streams to support future assessment efforts of MWCNTs. Such collaboration will serve to fortify connections between parallel research efforts (Schug et al., 2013) and the needs of risk assessors and risk managers (Hansen

and Baun, 2012; Schrurs and Lison, 2012). These connections are promoted by the CEA framework and process (Powers et al., 2012) and are essential if the scientific community is to move efficiently toward scientifically informed risk management of ENMs.

FUNDING

U.S. Environmental Protection Agency (EP-C-09-009, EP-W-09-004).

ACKNOWLEDGMENTS

We thank Harvey Clewell and Miyoung Yoon (the Hamner Institutes for Health Sciences) for their suggestions regarding research needs specific to PBPK modeling of MWCNTs. We are grateful to Amalia Turner and Kristen Fedak (ICF International) for their significant contributions to the case study document used to brief workshop participants. Christine Hendren and Khara Grieger (RTI International) were instrumental in the development and implementation of the workshop and collective judgment process. Appreciation is also expressed to Barbara Buckley, William Boyes, Lyle Burgoon, Reeder Sams, and John Vandenberg (U.S. Environmental Protection Agency) for providing a thorough review of the draft manuscript. The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

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