Meeting Summary Report
Material Characterization of Nanoscale Materials

(September 6 and 7, 2007
Peer Consultation Meeting)

U.S. Environmental Protection Agency
Office of Prevention, Pesticides and Toxic Substances
1200 Pennsylvania Avenue, NW
Washington, D.C. 20460

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1.0   INTRODUCTION

This report summarizes the Panel discussions and public comments during the Peer Consultation Public Meeting on The Material Characterization of Nanoscale Materials organized by the U.S. Environmental Protection Agency (EPA). The meeting took place in Arlington, VA, on September 6 and 7, 2007. Eastern Research Group, Inc. (ERG), a contractor to EPA, organized the logistics, provided facilitation support, and prepared this summary report. Meeting minutes were not prepared and a transcript was not recorded. The intent of this report is to provide an overview of the discussion that occurred. No attempt has been made to analyze or evaluate any portion of the discussions. The discussion and comments presented in this summary reflect individual opinions of the commenters and should not be considered to be the opinion or belief of EPA.

1.1   <u>Panel Members</u>

- Dr. Joseph BelBruno, Professor, Department of Chemistry, Dartmouth College.

- Dr. Ahmed Busnaina, William Lincoln Smith Professor and Director, NSF Nanoscale Science and Engineering Center for High-rate Nanomanufactruing and the NSF IURC Center for Microcontamination Control, Northesastern University.

- Dr. Richard Canady, DABT, Office of the Commissioner, Food and Drug Administration.

- Dr. Alan Cassell, Project Scientist/Task Manager, Ames Research Center, National Aeronautics and Space Association.

- Dr. Vicki Colvin, Director, Center for Biological and Environmental Nanotechnology (CBEN), Rice University (participated on Day 2, September 7th only).

- Dr. Joerg Lahann, Professor, Department of Chemical Engineering, University of Michigan (participated on Day 1, September 6th only).

- Dr. Vladimir Murashov, Special Assistant to the Director, National Institute for Occupational Safety and Health (NIOSH).

- Dr. James Murday, Associate Director for Physical Sciences, Office of Research Advancement, University of Sourthern California.

- Dr. John Small, Group Leader, Center for Nanoscale Science and Technology, National Institutre of Standards and Technology (NIST).

- Dr. David Warheit, DuPont Haskell Laboratory.

1.2   <u>Meeting Background and Purpose</u>

As part of EPA's initiative to address growing interest in the potential health and safety issues of engineered nanoscale materials (NMs), EPA is developing the voluntary Nanoscale Materials Stewardship Program (NMSP). This program is being designed to encourage responsible commercial development of NMs. The NMSP will enable EPA, the relevant industry, and interested stakeholders to better assess potential risks to human health and the environment from NMs and to identify risk management practices which may reduce such potential risks.

The NMSP is intended to include but is not limited to engineered NMs manufactured or imported for commercial purposes as defined in 40 CFR 720.3 (r). The NMSP is envisioned to have two parts: 1) a basic reporting program and 2) an in-depth program. This public peer consultation meeting is intended to support the NMSP by identifying material characterization data that participants could submit under the Basic Program if they are known or are reasonably ascertainable as defined in 40 CFR 720.3 (p). The data and experience generated by the basic reporting phase will help to inform the types of in-depth data to be developed.

The Scientific Peer Consultation Public Meeting on The Material Characterization of Nanoscale Materials was organized to help clarify which data and elements should be included in the NMSP basic and/or in-depth program (see Appendix A for the meeting agenda). The goal was to have an applied discussion that considers 1) the current understanding of material characterization as it relates to NMs and 2) how this understanding can be used to guide the Agency's thinking regarding the material characterization data elements that would be most useful and important to include in the NMSP

Prior to the meeting, EPA developed a paper to facilitate discussion on topics related to material characterization (Appendix B). EPA then convened a Panel to review the discussion paper. The Panelists reviewed the discussion paper and submitted comments prior to the meeting, which were summarized and circulated for review to aid the meeting discussions (Appendix C). The requested approach for the Panel during the meeting was to discuss what data are known or reasonably ascertainable to characterize NMs. This was then to be followed by a discussion on the methodology used to obtain and use characterization endpoints of interest. There was no attempt prior to, or during the meeting, to arrive at joint decisions, reach consensus, or provide majority advice.

The meeting was organized into a series of sequential sessions over a two-day period. After initial introductions from EPA, an open, facilitated discussion occurred regarding the questions posed to the Panel in the topical areas per the discussion paper. After Panelists concluded their discussion, meeting observers were asked to provide comment. As time permitted, the Panel then responded or continued discussion based on the public comments.

Additional information pertaining to the specific charge to the Panel, a summary of their written responses prior to the meeting, and the meeting agenda can be found in the public docket and appendices of this report

2.0   DAY ONE SUMMARY (SEPTEMBER 6, 2007)

2.1   <u>Introductions</u>

Opening Remarks and Background

Jim Alwood (EPA, Office of Pollution Prevention and Toxics (OPPT), Chemical Control Division). Mr. Alwood greeted Panelists and members of the public. Mr. Alwood stated that the purpose of the meeting was to solicit input from individual Panelists and the public. EPA would then consider all input, but there would be no specific attempt during the meeting to come to consensus on any discussion topics.

Greg Fritz (EPA, OPPT, Economics, Exposure, and Technology Division (EETD)). Dr. Fritz addressed the group and summarized the scope of the meeting. He stated that the Panelists had been asked to review a document titled, "*Peer Consultation Public Meeting on The Material Characterization of Nanoscale Materials: Discussion Topics, August 7, 2007*" prior to the meeting and consider the corresponding series of questions. Dr. Fritz stated that the Panel discussions surrounding the questions during the meeting would help inform EPA as the Nanoscale Materials Stewardship Program (NMSP) is structured, specifically regarding the data elements that may be requested for participation in the basic and in-depth programs.

Dr. Fritz then summarized the content of the discussion paper and the specific questions that EPA asked the Panelists.

Nanoscale Materials Stewardship Program Overview and Status

Jim Willis (EPA, Office of Pollution Prevention and Toxics; Director, Chemical Control Division). Mr. Willis provided additional remarks to set the stage for the meeting, including a background and history on EPA's effort to develop the NMSP and how material characterization fits into the program.

He stated EPA's intent is to develop and implement the NMSP in an open and transparent manner. The meeting is the third in a series of public meetings held to solicit input on various components of The Program. The first was held on October 19th and 20th, 2006 regarding Risk Management Practices; and the second was held August 2nd, 2007 to discuss EPA's, "Concept Paper for the Nanoscale Materials Stewardship Program under TSCA" and the "TSCA Inventory Status of Nanoscale Substances - General Approach".

Mr. Willis also discussed the overall status of the NMSP. EPA is currently in the "design phase" of the program. The current meeting is intended to help finalize the structure and design. After completion of this first phase, the NMSP will be implemented such that participants will submit information to EPA. After the "implementation phase" has been functional for a time period (still to be determined), an "evaluation phase" will begin. During the evaluation, EPA will review data submitted, modify the program as appropriate, and provide a summary to the public.

Facilitated Introductions and Charge to Panelists

Jan Connery (ERG) facilitated the meeting. The Panelists were first asked to briefly introduce themselves. After introductions, Ms. Connery stressed to all participants and observers that there would be no attempt to reach consensus during the discussion. Rather, the intent of the meeting is to provide EPA with input from a broad, scientific stakeholder community on the various discussion topics. All comments and suggestions were to be recorded as individual opinions for EPA to consider while proceeding with development of the NMSP. However, to aid EPA's evaluation, the meeting summary document would note instances when multiple Panelists concurred on any topic.

Ms. Connery then noted six general topical areas that were not intended for discussion during the meeting.

- EPA Policy
- Classification and Nomenclature
- Environmental Fate or Transport
- Risk Management or Hazard Assessment
- Solutions to Perceived Data Gaps
- Assessment of the NMSP or other EPA programs

Dr. Canady asked for clarification regarding the topic of nomenclature.

Tracy Williamson (EPA, OPPT, EETD; Chief, Industrial Chemistry Branch) provided a response. Dr. Williamson agreed that a standardized classification scheme and standardized nomenclature are important and relevant. However, she noted that there are ongoing efforts to discuss these topics in great detail. Therefore, EPA desired not to devote significant portions of the Material Characterization meeting to address nomenclature, recognizing that there may be some topics that require some discussion.

Public Comment and Q/A Session

Public Comment #1: Scott Sweeney (Safer Nanomaterials and Nanomanufacturing Initiative, SNNI) provided a prepared statement that included three primary points, briefly summarized below.

1. He noted his organization has developed a library with information pertaining to nanomaterials and suggested this type of repository may be very useful.
2. He noted that the purity of nanomaterials is important and applicable to environmental assessments. He also provided examples of test data that may show purity has a considerable toxicological impact.
3. His organization has access to "rapid characterization methods" to evaluate purity, noting that electron microscopy has been used historically; but it is not useful data in many circumstances. Mr. Sweeney suggested the use of other techniques such as nuclear magnetic resonance spectroscopy (NMR), Thermogravimetric Analysis (TGA), and X-ray photoelectron spectroscopy (XPS).

Mr. Sweeney's statements resulted in extensive dialogue from Panelists regarding the test data and SNNI's characterization methods. Mr. Sweeney stated SNNI is developing a data base that could be made available upon request. Mr. Sweeney was asked to provide written comments to EPA.

Public Comment #2: Shekhar Subramoney (DuPont Company) asked EPA to clarify the rationale for excluding discussions regarding hazard assessment from this meeting.

Dr. Williamson noted that NPPTAC recommended a public meeting specifically devoted to material characterization, which she acknowledged helps inform risk management and hazard assessments. Dr. Williamson also reminded the audience of a previous meeting (held October 19th and 20th, 2006) that was focused on the Risk Management of Nanomaterials. Portions of that meeting were specifically devoted to discussing hazard assessments.

Dr. Williamson also clarified that EPA was interested in all media (e.g., dry state, air, water) regarding material characterization.

Public Comment #3: Shaun Clancy (American Chemistry Council, ACC)) requested that EPA clearly state the test methods that will be required of NMSP participants to provide appropriate data when the NMSP is ultimately developed.

2.2 <u>General Discussion</u>

Ms. Connery asked each Panelist to provide their general thoughts and comments on the discussion paper and issues surrounding material characterization of nanomaterials before delving into the detailed session topics.

Dr. Warheit commented that the questions posed in the discussion paper were generally appropriate. He also noted that from a toxicological view, many people have attempted to generate lists of appropriate tests for characterizing nanomaterials. His view is that this exercise typically results in an extensive list that is too long because a great number of tests will provide potentially-useful information. One reason is the nearly infinite number of potential nanomaterials and a corresponding infinite number of uses. He suggested developing a smaller, prioritized list of required information and the tests that are needed to provide that information. Dr. Warheit provided a suggested list in his pre-meeting comments.

Dr. Warheit also stated his belief that purity of the nanomaterial being evaluated is very important.

Finally, Dr. Warheit discussed his view that exposure assessments of nanomaterials are highly dependent on:

- Whether the material is in a wet or dry state;
- The composition of the matrix being evaluated; and,
- Whether assessments are conducted on the core or the shell of the nanomaterial

Dr. Canady asked for clarification from EPA regarding the rationale for establishing the categories that were posed as starting points for grouping nanomaterials in the discussion paper.

Dr. Williamson responded that the categories were established to facilitate discussion and that they could be altered if appropriate. After additional discussion, Dr. Williamson acknowledged that the Toxic Substances Control Act (TSCA) does not include categories of chemicals on the TSCA Inventory. She noted, however, that categorizing chemicals sometimes aids EPA's internal review process when determining how to assess and evaluate chemicals. Therefore, it may be useful to establish categories.

Dr. Busnaina noted that he would have suggested different categories because the existing categories were developed for macro materials based on the distinguishing characteristics of their properties. He stated that these characteristics are not appropriate for distinguishing between different nanomaterials. Dr. Busnaina suggested developing categories based on: chemicals with inert surfaces, active surfaces, and functionalized surfaces.

Dr. Busnaina also stated that EPA should recognize qualifying terminology such as "simple structures" may not be appropriate. As an example, he noted that even discussing nanoscale titanium dioxide can be very complex.

Dr. Murday initiated a clarifying discussion regarding composites that include a macromaterial core and a nanomaterial attached to the surface.

Dr. Williamson stated the group may not want to exclude these materials, but she suggested the discussion should focus on free-standing nanomaterials.

2.3 Session 1: Discussion – Types of Nanoscale Materials & Their Structure and Chemical Composition

Ms. Connery opened this session by stating that there were five related questions in EPA's discussion paper that would be discussed (questions 1, 2, 3, 12, and 13). She reviewed each question and then asked Panelists to discuss their responses. The questions and subsequent discussions are presented below.

2.3.1 Question 1: Are there any other significant categories, based on structure and chemical composition, that should be included in this discussion because they are substantially different from the categories mentioned (e.g., hybrids, self- assembly devices, others)?

Dr. Murday suggested that using the term "molecule" when discussing nanomaterials may not be appropriate. He commented that this term implies uniformity, whereas many nanomaterials are not uniform and are not based on a specific chemical formula (for example, the number of atoms in a gold nanomaterial is flexible). Dr. Murday agreed with the comments provided by Dr. Murashov in his pre-meeting comments.

Dr. Murashov reiterated the statements made in his pre-meeting comments. He noted a particular concern regarding "purity". He noted that if a nanomaterial were defined based on a chemical structure, EPA would need to determine an acceptable "level of purity". For example, EPA would need to specify a range regarding the number of atoms for various nanomaterials.

Dr. Murashov also suggested that EPA should consider the difference in chemical composition between the core and the surface of the nanomaterial (and the associated purity of these two different regions of the nanomaterial). He noted that this is important because the effects based on the content of the core are different than those based on the surface. The surface chemistry may result in more immediate concerns related to exposure; however, the potential hazards associated with the core are also important because the surface may eventually degrade.

Dr. Murashov suggested that if EPA must develop categories and corresponding characterization, the Agency could group nanomaterials based on the structure of the core, recognizing surface chemistry and function will have effects.

Dr. Murashov pointed out inconsistencies in the proposed grouping into categories of nanomaterials described as "organic" and "inorganic". He suggested "carbonaceous" is a more suitable category to describe carbon-based nanomaterials, noting that pure carbon is not "organic".

Dr. Canady reviewed his written, pre-meeting comments. He then expanded on a few topics. First, Dr. Canady recognized the need to develop categories. However, he proposed that EPA recognize that the categories could be modified in the future as more information is acquired (possibly modifying the definition of existing categories, or adding and deleting categories).

Dr. Canady suggested EPA consider nanomaterials that may exist as "combinations" of the existing categories (he suggested some could fall into multiple categories as they are written).

Dr. Canady also suggested a separate category for emulsions, vesicles, and micelles. He stated they are omitted from the categories in EPA's discussion paper; however, there are drug applications that may eventually be utilized in industry.

Dr. Joerg Lahann agreed with Dr. Canady's comments regarding emulsions, vesicles, and micelles. He also agreed with the comment regarding the potential for "combinations", and suggested development of a "hybrid" category (or categories, if appropriate).

Dr. Lahann stated that the effects of nanomaterials are often dependent upon functionality; not their specific size, structure, or composition.

Dr. BelBruno commented that it is appropriate to develop initial categories based on the chemical structure; however, functionality is very important and should be considered for future categorization.

Dr. Cassell stated that organometallic materials should be a separate category. This initiated a discussion regarding the potential effects from a metal atom that is surrounded by

various functional groups. *Various Panelists* noted potential concerns depending on the specific metal and the functional groups.

Dr. Canady suggested that organometallic materials could be a sub-category or could be a "hybrid" as previously discussed.

Dr. Murday suggested the following categories and subcategories:

- Free-Standing nanomaterials
    - Materials whose core is always capped
    - Materials whose core consists of a uniform shell (e.g., quantum dots)
    - Hybrid materials
        - those with multiple shells
        - those with multiple nanostructures linked together
- Nanomaterials embedded in a composite material
- Thin-film nanostructures (e.g., those used in electronic devices)

Dr. Murday suggested this categorization would allow analysis in a hierarchy from simple to more complex.

Dr. Lahann agreed with Dr. Murday's proposed categories.

Dr. Murashov also agreed.

EPA Response, Public Comment, and Q/A Session

Public Comment #1: Dan Ewart (NanoProducts Corp.) noted that TSCA is based on chemical structures. Therefore, he commented the EPA may need to consider implications of grouping nanomaterials in categories that are not based on chemical formulae.

Dr. Williamson reiterated her previous remarks that categories of chemical are not included on the TSCA inventory, but EPA utilizes categories (based on structure) to assist internal review.

She also presented a brief overview of EPA's current vision for the NMSP. This vision includes the need to understand how to characterize nanomaterials based on chemical identification information and physical-chemical properties and the test methods needed to acquire the characterization data. Dr. Williamson recognized that the list of properties and tests may be lengthy; therefore, EPA is hoping to receive input on how to prioritize the most important, needed information.

Public Comment #2: Mark Herwig (General Electric) asked if other organizations had already developed categories for nanomaterials. This question resulted in significant discussion regarding other categorization attempts.

Mr. Willis specifically discussed current activities of the Organization of Economic Cooperation and Development (OECD) Working Party on Manufactured Nanomaterials and of

the International Organization for Standardization (ISO) Technical Committee (TC) that promotes international standardization in nanotechnology (TC229).

Dr. Canady reiterated his view that it is appropriate to establish categories for initial discussion and evaluation. However, EPA should allow for category modification as more information becomes available.

2.3.2 Question 2: For the different categories of nanoscale materials, what is the current state of knowledge about structure and chemical composition?

Dr. Small indicated that the structure and composition of some nanomaterials is known and can be measured "very accurately." However, a problem occurs when nanomaterials are purchased from manufacturers. He noted that the actual composition, core, and sometimes functional groups vary from batch to batch; even when product specifications are well-defined.

Other Panelists agreed and brought up this as an issue during several discussions throughout the remainder of the meeting.

Dr. Warheit commented that from a toxicological perspective the structure and composition is well understood for specific nanomaterial-types that have been studied; however, this is not necessarily true for nanomaterials as a whole.

Dr. BelBruno agreed with and expanded upon Dr. Small's assertion that there are differences between batches of the same material. He noted that given a sample, laboratories can conduct tests to characterize the nanomaterial. However, the results may only apply to that particular batch.

Dr. Murashov stated that there are only two examples of nanomaterials he feels are well-characterized (fullerenes and dendrimers). He noted that even titanium dioxide has multiple states and is not completely characterized.

Dr. Busnaina commented that it may not be possible to acquire all the information needed to fully characterize each nanomaterial from a particular supplier. He questioned whether the suggested categories are appropriate. Dr. Busnaina then suggested selecting a few properties to prioritize the characterization (such as surface activity) then conduct follow-up evaluations with secondary properties (such as oxidation potential).

Dr. Murday commented that nanomaterials are very complex; therefore, the proposed properties for characterization may not apply. For example, he noted that the core is often very different in its chemistry and functionality than the surface. Therefore, he suggested it may be more appropriate to consider properties of concern rather than the actual chemical structure.

Dr. Murashov agreed with Dr. Murday's previous comment. He also agreed with Dr. Small's comment about variability between batches.

Dr. Lahann suggested that although there may be variability between batches, there may be appropriate ways to characterize the purity based on statistical analyses. He commented on current methods of characterizing uncertainty when evaluating the purity of polymers.

Dr. Canady observed that based on other Panelist's comments regarding this question it seemed there is some level of knowledge regarding certain specific nanomaterials; however, it is not appropriate to state there is significant knowledge for nanomaterials in general and there is no knowledge for most.

EPA Response, Public Comment, and Q/A Session

Dr. Williamson provided comments on the Panel discussions. She stated the discussion was very informative and asked Panelists and observers to provide additional information regarding the variability between batches. Dr. Williamson also asked Panelists to elaborate on the specific characteristics being studied (e.g. for metal oxides). Finally, she asked if there are certain characteristics that are more important than others for characterization. The following summary of discussions ensued.

Dr. Murashov indicated that X-Ray crystallography is commonly conducted on metal oxides.

Dr. Warheit agreed with Dr. Murashov, but noted that this was typically done to evaluate the core. He noted there are different grades of TiO2 for which his laboratory had tested (and published information). His work considered different formulations for first time (as far as he was aware). He also stated there are different degrees of surface coatings, which may not be common knowledge.

Dr. Murashov addressed another question from Dr. Williamson by stating he felt primary data needs to include a measurement of the distribution of material in the sample, the nanomaterial's size, and its shape.

Dr. Fritz asked Panelists to elaborate on their experience with variability between samples.

Dr. Busnaina confirmed this was a significant issue, elaborating on his specific experiences. For example, he observed considerable variability when purchasing nanomaterials with the same product specifications from the same supplier. He specifically noted that processing steps that a manufacturer believes are routine can change certain properties and chemical characteristics of nanomaterials.

Dr Murashov then initiated a discussion regarding "surface activity". He commented that although surface activity is an important consideration, it was not clear how to define or measure this characteristic (e.g., redox potential or something else?).

Dr. Fritz asked Panelists to elaborate on potential differences or concerns regarding tests conducted on dry nanomaterials vs. emulsions.

Dr. Warheit initiated an extended discussion among multiple Panelists and EPA representatives on the importance in recognizing differences in nanomaterials in dry vs. wet matrices. Differences will include the toxicological and environmental effects as well as the most important characteristics to measure the effects and the corresponding measurement techniques.

Public Comment #1: Shekhar Subramoney (DuPont Company) commented that his experience is that analytical tools are available to identify the structure and composition of nanomaterials. He agreed there is variability between batches, but disagreed with most Panelists' assertion that this is a primary concern.

2.3.3 Question 3: Can structures and chemical composition be correlated to specific properties and is this correlation quantifiable? Chemical Identification Elements (e.g., which ones? for which substances? test methods?)

Dr. Warheit referenced a paper that was recently published on the lung toxicity of pristine and fully hydroxylated fullerenes. The original *in vitro* study showed increases in toxicity of at least three orders of magnitude between the pristine (more toxic) and the fully hydroxylated fullerene samples. However, in the *in vivo* pulmonary toxicity study, few toxicity differences were measured between the two samples. The different results between the two studies indicate that the structure and chemistry and other *in vitro* analyses may not be predictive of the inherent toxicity of the nanomaterial and that each sample has to be evaluated on a case-by-case basis.

Dr. Murday felt there is a correlation between structures and chemical composition, and properties. He also felt computer modeling based on structure and composition could be developed to predict properties. Dr. Murday also suggested that EPA contact industry stakeholders that produce catalysts regarding their work on this topic. Finally, Dr. Murday noted that we still do not fully understand everything that impacts a living system, whether or not the material is nanoscale. This may affect correlations.

Dr. Murashov referred to his pre-meeting comments, reiterating them and stating he felt there were correlations.

Dr. Lahann also agreed it is possible to develop models and correlations once data are obtained. However, he noted there are still many unknowns.

Additional discussion between Panelists followed these specific remarks. *Multiple Panelists* stated that theoretical modeling could be conducted; however, the model goals would need to be clearly defined. Further, *Panelists* acknowledged modeling results would need to be validated and tested for scale-up to production levels.

2.3.4   Question 12:   How important are impurity identity and impurity levels to the understanding and characterization of nanoscale materials?

Dr. Cassell replied that the effects of impurities are extremely important. However, he noted examples where the impurities result in more risk than the nanomaterial itself (e.g., when heavy metals are used as catalysts). Dr. Cassell also stated that the term "impurity" must be defined when referring to nanomaterials (referencing previous discussions on this topic). He suggested that small changes in structure, aspect ratio, and location of ligands may not result in different chemical formulae, but will result in very different characteristics. These could be considered impurities.

Dr. Murashov agreed with these comments. He noted that it is important to agree on what is considered the "pure material" before an evaluation of impurities can be conducted. Further, Dr. Murashov suggested establishing an acceptable range for a material to be considered "pure".

Dr. Small noted that measuring purity may be difficult (depending on the characteristic that is selected to define purity). He also noted that tests must be designed to distinguish between the bulk material and the catalysts within it.

Dr. Small's comment regarding catalysts resulted in an extended discussion about what other chemicals in the commercial nanomaterial product should be evaluated (besides the nanomaterial itself). *Panelists* noted examples where specific catalysts are expected to remain with the nanomaterial and other examples where contamination of unknown chemicals results from processing operations. *Panelists* suggested this is of primary concern during full-scale production opposed to Research & Development where samples are typically tested and monitored more closely. *Multiple Panelists* suggested the focus should be on characteristics that can impact environmental health and safety.

EPA Response, Public Comment, and Q/A Session

Dr. Williamson asked the Panelists and observers if EPA should ask for more information on impurities for the NMSP.

Public Comment #1: Shaun Clancy (ACC) stated that the issue of impurities may be very important in context of the NMSP. He recognized that EPA may request data on specific chemical impurities in the commercial mixture. He also recognized the potential for multiple NMSP participants to submit data on the same nanomaterial. Test results may be affected by impurities; therefore, concentration data and data regarding the specific impurities will be needed to compare the results.

Public Comment #2: Donald Ewert (NanoProducts Corp.) commented that his customers require information regarding impurities. Therefore, he believes this is an important topic. He further stated that his company routinely characterizes their products and the corresponding impurities, believing that measurement techniques are available to acquire appropriate information.

2.3.5   **Question 13: Are there routine purification procedures that can effectively control or remove impurities, when desirable, for certain classes of nanoscale materials?**

Dr. Canady responded that these procedures are available. However, they vary depending on the specific nanomaterial. There is not one procedure that is universally suitable.

Dr. Cassell referred to his pre-meeting comments for more detail. He also specifically noted that the methods currently used for nanomaterials are the same as those used for macromaterials.

Each Panelist was asked for input and all concurred with the statements made by Dr. Canady and Dr. Cassell.

2.4   **Session 2- Discussion: Physical Chemical Properties**

Ms. Connery opened this session by stating that there were five related questions in EPA's discussion paper that would be discussed (questions 4 through 8). She reviewed each question and then asked Panelists to discuss their responses. The questions and subsequent discussions are presented below.

2.4.1   **Question 4:   Which physical-chemical properties are relevant to nanoscale materials and how? Which are known or reasonably ascertainable and which have data gaps?**

Prior to the discussion on this topic, *Dr. Williamson* clarified that relevant properties include those that may impact the function of the material in its intended use, or those that may impact the material's effects.

Dr. Murday commented that virtually all properties are relevant to some degree; however, he suggested some have a more dramatic influence. Dr. Murday referred to his pre-meeting comments for a list of those he believes are most significant.

Dr. Murashov agreed with Dr. Murday's list but suggested a few additions, referenced in his pre-meeting comments for a list of the most relevant properties.

Dr Warheit reminded the group of the earlier discussion that the biological matrix from which the nanoparticle is dispersed can significantly affect characteristics (e.g., wet (water, culture media) or dry).

Dr. Lahann commented that EPA should try to identify properties that are unique to nanomaterials; however, he did not suggest what those properties may be.

Dr. Canady noted that there are differences in observed properties, and the corresponding characteristics, when the nanomaterial is aggregated.

Dr. Busnaina agreed with the lists that were provided by Dr. Murday and Dr. Murashov, and with the discussion regarding aggregates. He added that surface energy is also an important property (recognizing it is not easy to measure).

Dr. BelBruno agreed with the lists discussed above.

EPA Response, Public Comment, and Q/A Session

Dr. Fritz asked Panelists to expand on comments regarding agglomeration, specifically asking whether surface energy was a factor in agglomeration.

Various Panelists commented that surface energy (or perhaps "interfacial energy") does affect the agglomeration potential, but the degree is case-by-case. Also, *some Panelists* noted that nanomaterials can be coated to prevent agglomeration. This is desirable in some situations and can be done to reduce the hazard. However, *other Panelists* noted that coatings do not fully mitigate concerns.

Public Comment #1: An observer asked the group if there are concerns related to silver in the nanoscale that are different from those in the macroscale. Panelists did not provide a response.

Public Comment #2: Shaun Clancy (ACC) suggested that EPA should revise their investigation on this topic to only include an analysis of properties that are important to specified toxicological end points.

Mr. Clancy also urged EPA to develop the required elements for the NMSP based on information that will help fill data needs (information that will inform a risk assessment opposed to general data gaps).

Public Comment #3: Steve Hayes (Gobbell Hays Partners, Inc.) suggested that EPA should revise their investigation on this topic to focus on properties that are relevant:

- Compared to materials in the bulk form
- Performance
- For toxicological effects
- For fate & transport analyses
- Persistence (because it relates to considerations during cleanup)

He also suggested that EPA consider topics that may affect clean up of unintended releases (e.g., spills).

Public Comment #4: Tanya Spellman (Washington Council of Government) commented that EPA should consider the ultimate fate of nanomaterials; specifically noting that they may be

transferred to Publicly–Owned Treatment Works and these facilities will need to understand appropriate handling and cleanup procedures. Ms. Spellman also concurred with Mr. Clancy's remarks that EPA should only request information that will help inform a risk assessment.

2.4.2   Question 5:   Are there properties that would have little or no relevance under the NMSP?

Multiple Panelists discussed their view that virtually all properties have some importance. *Some Panelists* commented that because there is a lack of information regarding potential effects associated with exposure to nanomaterials, EPA should attempt to acquire as much information as possible rather than limiting the effort to investigate certain properties. *Other Panelists* acknowledged that all properties could potentially result in an impact; however, they felt it would be more appropriate to prioritize efforts and focus on properties that are most likely to result in an impact. They acknowledged that the costs associated with testing may have an impact on which tests should be performed, if only limited useful information would be produced.

Multiple Panelists suggested specific properties that may not be relevant for certain aspects of a risk evaluation. These are briefly summarized below.

Dr. Murashov referred to his pre-meeting comments for specific suggestions. During the discussion he suggested that refraction may not be relevant.

Dr. Lahann commented that elastic properties may affect cell uptake of nanomaterials; therefore, they could be relevant.

Dr. Murday agreed with Dr. Murashov's list in general but stated that optical properties can sometimes be important.

Dr. Warheit reiterated his previous statement that virtually all properties have some relevance.

Thermal properties and superconductivity were also specifically mentioned as properties that may not be relevant.

EPA Response, Public Comment, and Q/A Session

There were no public comments or questions.

2.4.3   Question 6:   Which properties are associated with aggregated or agglomerated nanoscale materials, as opposed to properties that are inherent to the material regardless of physical form?

Dr. Murashov reviewed his pre-meeting comments. He stressed that biological activity can be affected by agglomeration.

Dr. Small stated that agglomeration affects the fate and transport of nanomaterials in the environment.

Other Panelists agreed with Dr. Murashov's initial comments and the subsequent discussion that ensued during his review. It was noted that larger aggregated particles are easier to remove from a sample by filtration.

EPA Response, Public Comment, and Q/A Session

There were no public comments or questions.

2.4.4   Question 7:   Are there routine manipulations of nanoscale materials that result in physical-chemical properties changes or other defining characteristics (e.g., surface modifications of nanotubes to enhance solvent dispersibility)?

EPA added a clarifying example: Coatings can be applied to minimize agglomeration. How are these applied?

Multiple Panelists indicated there are many processes that industry uses, but each is developed and implemented case-by-case, depending upon the specific situation. *Multiple Panelists* indicated there is not a "routine" process. However, Panelists offered the specific examples presented below.

Dr. Warheit stated that sonication can reduce agglomeration.

Dr. Murday stated that the surface of many nanomaterials must be "capped" during production. Depending on the complexity of the material and the end use, the cap may include a ligand that imparts new functionality.

Dr. Cassell stated there are many processes that manufacturers use to package materials for transport to customers. These include annealing and application of coatings. He referred to his pre-meeting comments for additional information.

EPA Response, Public Comment, and Q/A Session

Public Comment #1: Mark Herwig (GE) stated that routine manipulations include applying coatings to alter the functionality of the nanomaterial. However, the type of coating and desired functionality affects the specific processes.

3.0   DAY TWO SUMMARY (SEPTEMBER 7, 2007)

3.1   Session 2- Discussion: Physical Chemical Properties (Continued)

The meeting was reconvened on September 7th, with *Ms. Connery* reminding Panelists and observers that Day One concluded with a discussion pertaining to Question 7 from the discussion paper. Ms. Connery then asked Panelists to continue their efforts beginning with Question 8.

3.1.1   Question 8:   How should physical-chemical property data be prioritized for the NMSP? Based on availability, effect (toxicity or exposure criteria), or other factors?

Dr. Warheit stated that there is a very long list of physical-chemical data that may be useful. However, he provided a list of "minimal essential criteria" in his pre-meeting comments. *All Panelists* agreed in general with Dr. Warheit's list (with some specific comments as listed below).

Dr. Warheit then provided some additional points for EPA to consider when finalizing the list:

- Particle size
- Wet vs. dry state
- Differences in the core vs. the shell (the surface is often where most activity occurs)

Dr. Warheit also referred EPA to five studies that indicate the surface activity is often more important than the particle size (see his pre-meeting comments).

Dr. Canady agreed with Dr. Warheit's list and comments. However, he noted that the list focuses on data to inform a hazard assessment. Additional data may help inform other environmental effects that have delayed or indirect effects on humans or ecosystems, for example, as are recognized in the ozone depletion and global warming issues. He also noted that the current Panel did not include an expert on ecotoxicity. Additional items may be important from an ecotoxicity perspective.

Dr. Colvin concurred with Dr. Warheit's list; however, she suggested acquiring all data and information would be useful (rather than limiting the effort to a prioritized list). *Other Panelists* agreed that it may not be appropriate to "eliminate" certain data elements and that acquiring as much information as possible may be appropriate.

Multiple Panelists agreed that surface reactivity is very important. An extended discussion regarding surface reactivity, the type of information needed to appropriately define it, and the corresponding tests that would be required ensued without specific resolution. The types of chemical reactions associated with the surface were discussed. In addition, surface reactivity was related to hazard and biological activity. *One Panelist* encouraged EPA to consult NIH on this topic.

Dr. Busnaina commented that EPA should recognize that Confidential Business Information (CBI) concerns may make it difficult to acquire this information.

EPA Response, Public Comment, and Q/A Session

Public Comment #1: Andy Atkinson (Environment Canada) initiated a discussion by asking if agglomeration impacts the proposed list of primary properties to consider. *Multiple Panelists* stated agglomeration would affect the properties (particularly surface reactivity). Follow-up discussions resulted in a suggestion to add a study of persistence because agglomeration affects persistence in the environment (and there is the potential for agglomerated nanomaterials to disagglomerate over time).

3.2   Session 3 - Discussion: Nanoscale Manufacture and Processing

Ms. Connery opened this session by stating that there were three related questions in EPA's discussion paper that would be discussed (questions 9, 10, and 11). She reviewed each question and then asked Panelists to discuss their responses. The questions and subsequent discussions are presented below.

3.2.1   Question 9:   What are the common processes used to manufacture nanoscale materials?

Dr. Colvin referred to a recent workshop devoted to this specific topic. She stated the summary will be available soon. In general, participants developed three primary methods for each of which there are many specific processes:

1. Gas phase processes
2. Liquid phase processes
3. Top-down manufacturing

Dr. Colvin noted agglomeration concerns are relevant to methods (1) and (2) and that coatings are applied to prevent agglomeration for type (2).

Dr. Murashov suggested that biological processes leading to the production of nanomaterials, such as nanoscale metal oxide particles, could be identified as a major distinct method. This method can produce nanomaterials with unique compositions, properties, and impurities and therefore can be characterized by very unique hazard properties.

Dr. Murday commented that the above discussion refers to chemical-based processes. He noted that physical-based processes such as crushing and grinding are also commonly used.

Dr. Cassell suggested the following processes:

• Chemical vapor deposition

- Laser ablation
- Thin film deposition
- Self assembly (there could be "hundreds" of specific techniques

Dr. Murday indicted that various metallurgical processes using mechanical deformation are used.

EPA Response, Public Comment, and Q/A Session

Comment #1: Shekhar Subramoney (DuPont Company) stated he is aware of a large-scale production process (thousands of pounds per year) using mechanical/chemical exfoliation.

3.2.2   Question 10: How are processes used to produce specific characteristics or properties?

Dr. Cassell commented that chemical vapor deposition can be used to form one dimensional linear materials where the reaction time can be set to control length. Also, self-assembly techniques (with micelle-based approaches) can be used to control diameter. Finally, annealing can be used to modify the surface area and porosity.

Dr. BelBruno indicated that "normal chemical methods" are used for liquid synthesis.

Dr. *Murday* stated that there are different processes used for the following three steps:

1. Manufacture the core
2. Cap the core
3. Add a functionalized shell

This statement resulted in a discussion as to whether these general steps are typical. *Multiple Panelists* indicated these are typical and presented some examples.

EPA Response, Public Comment, and Q/A Session

There were no comments relevant to this topic.

3.2.3   Question 11: Which methods reduce particle size but do not result in property changes? Which methods reduce particle size and result in property changes?

Multiple Panelists asked for clarification from EPA. During the resulting discussion the Panelists and EPA agreed that the question was answered to EPA's satisfaction during discussions of previous questions.

3.3   <u>Session 4 - Discussion: Methodologies for Characterizing Nanoscale Materials Analytical Methods</u>

Ms. Connery opened this session by stating that there were eight related questions in EPA's discussion paper that would be discussed (questions 14 through 21). She reviewed each question and then asked Panelists to discuss their responses. The questions and subsequent discussions are presented below.

3.3.1   **Question 14: Are validated methods available for the different categories of nanoscale materials?**

Dr. Colvin stated that 10 methods are currently being validated and another 15 are in the pre-validation phase. She referred to her pre-meeting comments for additional information.

Dr. Small stated that NIST is developing standards. He also noted that it is difficult to develop Standard Reference Materials.

Dr. Murashov commented that ISO is in the process of developing standards. He also noted that there are no current industrial hygiene methods to assess nanomateials and suggested that some bulk-phase methods may be applicable (recognizing that further validation would be required).

Dr. Murday commented that equipment and tools are available and are used to measure and characterize nanomaterials on a regular basis; however, their use is not "validated". He also noted that certain analytical methodology (NMR & surface analytical tools) require "care" in interpreting data.

EPA Response, Public Comment, and Q/A Session

There were no public comments or questions.

3.3.2   **Question 15: Are there techniques that can be universally applied?**

All Panelists agreed that there are no techniques that can be universally applied. However, *several Panelists* provided specific examples of techniques that can be used in various situations. These examples are summarized below.

Dr. Murashov suggested that electron microscopy is widely used and accepted. *Multiple Panelists* agreed but noted some limitations. For example, *Dr. Small* commented that this technique works well to characterize the core, but not the shell of nanomaterials. Also, *Dr. Canady* noted that it does not provide statistical information (he referred to the previous discussions regarding the analysis of impurities).

Dr. Colvin provided EPA with citations for studies involving measurements of aerosols and indicated she would provide references.

Dr. Murday commented that there are other techniques that are available; however, many are cost-prohibitive.

EPA Response, Public Comment, and Q/A Session

There were no public comments or questions.

3.3.3   Question 16: For small quantities of materials, are there sampling, handling, and collection techniques as well as sample integrity, accuracy and precision QA/QC methodologies available?

Dr. Colvin referred to her pre-meeting comments for specific information. In addition, Dr. Colvin commented that she does not believe there is a need for nano-specific guidelines on this topic. She noted that temperature control is important (particularly when measuring and handling samples that may aggregate). She also stated that sonication can alter surface coatings and should be used appropriately.

Dr. Busnaina agreed with Dr. Colvin's comments, particularly regarding temperature control. He initiated a discussion between multiple Panelists where examples of problems when samples were shipped occurred due to extreme changes in temperature during flights to or from hot and cold geographic locations.

Multiple Panelists noted that best management practices for ultrafine materials may be appropriate for nanomaterials.

EPA Response, Public Comment, and Q/A Session

No comments on additional topics were provided.

3.3.4   Question 17: What is the status of standardization efforts? Are these efforts focused on broadly applicable characterization methods or category-specific methods?

Dr. Small commented that individual researchers develop and use their own standards (referring to standard reference materials). He also noted an upcoming NIST workshop that will be focused on developing a prioritized list of standard reference materials for NIST to develop.

Dr. Canady stated that OECD is also investigating this issue and may be developing a work group.

EPA Response, Public Comment, and Q/A Session

Public Comment #1: An observer asked for clarification regarding the phrase "NIST Certified" that he had observed on raw materials he had received from suppliers.

Dr. Small responded that, in general, this means that the specific sample can be traced to show it was compared to a NIST Standard Reference Material.

3.3.5   Question 18: What alternative or innovative methods or technologies can be applied to nanomaterial analysis?

Dr. Colvin suggested that a combination of mass spectroscopy and electro ionization could be appropriate.

Dr. Small indicated that helium ion microscopes may be appropriate to characterize materials greater than 10 nm.

Dr. Murday suggested that Atomic Force Microscopy (AFM) probes can be used to analyze individual particles.

EPA Response, Public Comment, and Q/A Session

Public Comment #1: Shekhar Subramoney (DuPont Company) stated that X-Ray absorption spectroscopy allows for analysis down to the atom-to-atom interactions. However, the equipment may be cost prohibitive.

3.3.6   Question 19: Are there models that are currently used to obtain property data for nanoscale materials? For which properties and which nanoscale materials?

Dr. Murashov referred to his pre-meeting comments for specific examples.

Dr. Cassell stated models exist to predict mechanical and electrical properties of carbon-based nanomaterial systems.

Multiple Panelists mentioned that models can be developed; however, they need to be specific for every situation. Therefore, there are no standard or general models. *Other Panelists* agreed they would have a concern regarding model output because it would be difficult to verify the results. Still, *some Panelists* agreed that appropriate models have been (or could be) developed for specific nanomaterials.

EPA Response, Public Comment, and Q/A Session

No comments on additional topics were provided.

3.3.7 **Question 20: Has any validation work been conducted that compares predicted values with measured data? For which properties and which nanoscale materials?**

Dr. Murday stated that some modeling had been conducted and verified but only for "simple" molecules; he was not aware of modeling for more "complex" molecules.

No other Panelists were aware of existing models that had been formally validated. However, *multiple Panelists* indicated there are efforts in academia to develop models. *Panelists* noted that these models may not be published because many scientific journals require validation and there are no accepted validation methods. *Dr. Cassell* stated one example is modeling of the thermoconductivity of carbon nanotubes that has been developed at the University of California at Berkeley.

EPA Response, Public Comment, and Q/A Session

No comments on additional topics were provided.

3.3.8 **Question 21: Are there current significant characterization needs for which the NMSP should investigate model development?**

Dr. Murashov reviewed his pre-meeting comments.

Dr. Warheit agreed with Dr. Murashov and added that accurate models generally cannot be developed until more information is acquired via data generation. *Dr. Busnaina* agreed.

Dr. Murday suggested that EPA coordinate with other organizations for development of specific types of models:

- Reactivity (consult industry's catalyst manufacturers)
- Toxicology (consult with NIH)

EPA Response, Public Comment, and Q/A Session

No comments on additional topics were provided.

3.4   <u>Session 5 - Discussion: Prioritization of Data Gaps</u>

Ms. Connery asked each Panelist to reflect upon the discussions that occurred during the meeting on each specific question and general topic, then identify the most important data gaps from their perspective. A summary list of Panelist responses is provided below.

Dr. Canady:
1. What materials will be developed in the future (5, 10, and 15 years from now)?
2. Specifically what needs to be validated?
3. What are the priorities for modeling?

Dr. Warheit:
1. How can surface reactivity be measured?
2. What would be the disparity between measuring in the wet and dry states for each particle type in each category?

Dr. Murday:
1. What parameters associated with health effects are most important?
2. What is the ability to get "reliable" validated measurements?
3. How can we determine the composition and structure of the shell of nanomaterials?

Dr. Busnaina:
1. There is a lack of data regarding surface reactivity (from a toxicological perspective).
2. There is a lack of data on functionalization (and effects from a toxicological perspective) specifically for commercial products.
3. What are the effects of impurities and the correlation to toxicity and properties (and at what concentration are they important)?

Dr. BelBruno:
1. There is a lack of information regarding the morphology (differences between the core, shell and crystal structures).
2. There is a lack of information regarding surface reactivity and correlated with #1 above.
3. There is a lack of information regarding impurities:
    a. How can consistency between batches be ensured?
    b. How are impurities defined?

Dr. Small:
1. How can surface reactivity be defined and measured?
2. There is a need to correlate data from wet vs. dry tests.
3. There is a data gap regarding the use of standards.
4. Determining criteria for categories.

Dr. Cassell:
1. Standardization.
2. Validation of characterization methods.
3. There is a need for a publicly-available information repository.
4. Surface reactivity.
5. Relationship between size and other properties.

Dr. Colvin:
1. There is a lack of understanding regarding wet nanoparticles and their interaction in the environment (micelles and complexation).
2. A mechanism for data mining and sharing of information is lacking.
3. Fully understanding the classification of commercial products

Dr. Murashov:
1. There is a need to harmonize and standardize:
   a. Common definitions (e.g., what does "impurity" mean?)
   b. A list of physical-chemical properties that are critical for a risk assessment.
   c. Protocol for collecting data.

3.5   <u>Closing Remarks</u>

After *Ms. Connery* confirmed that all Panelists had provided appropriate closing comments, EPA thanked the Panel and observers and the meeting was adjourned.

Appendix A

MEETING AGENDA

EPA United States
Environmental Protection Agency

Peer Consultation on the Material Characterization of Nanoscale Materials - Agenda

Holiday Inn Rosslyn at Key Bridge, Arlington, Virginia
September 6-7, 2007

DAY ONE: THURSDAY, SEPTEMBER 6

8:30 a.m.   Registration

9:00 a.m.   **EPA Opening Remarks and Background** .... *EPA*
  - Meeting Purpose and Objectives

9:30 a.m.   **Nanoscale Materials Stewardship Program (NMSP) Overview and Status**..... *EPA*
  - Summary of EPA's Concept for the NMSP
  - NMSP Relationship to OPPT TSCA Programs

10:00 a.m.   BREAK

10:15 a.m.   **Introductions, Meeting Agenda, Format and Charge**...................................*ERG*

10:45 a.m.   **Public Comment Period**..*ERG*

11:45 a.m.   LUNCH

1:00 p.m.   **Discussion: Types of Nanoscale Materials & Their Structure and Chemical Composition**
  - *Question 1: Are there any other significant categories, based on structure and chemical composition, that should be included in this discussion because they are substantially different from the categories mentioned (e.g., hybrids, self- assembly devices, others)?*
  - *Question 2: For the different categories of nanoscale materials, what is the current state of knowledge about structure and chemical composition?*
  - *Question 3: Can structures and chemical composition be correlated to specific properties and is this correlation quantifiable? Chemical Identification Elements (e.g., which ones? for which substances? test methods?)*
  - *Question 12: How important are impurity identity and impurity levels to the understanding and characterization of nanoscale materials?*
  - *Question 13: Are there routine purification procedures that can effectively control or remove impurities, when desirable, for certain classes of nanoscale materials?*

4:30 p.m.   Wrap-up and Prepare for Day Two

5:00 p.m.   ADJOURN

DAY TWO: FRIDAY, SEPTEMBER 7

8:30 a.m.   **Discussion: Physical-Chemical Properties**
- *Question 4: Which physical-chemical properties are relevant to nanoscale materials and how? Which are known or reasonably ascertainable and which have data gaps?*
- *Question 5: Are there properties that would have little or no relevance under the NMSP?*
- *Question 6: Which properties are associated with aggregated or agglomerated nanoscale materials, as opposed to properties that are inherent to the material regardless of physical form?*
- *Question 7: Are there routine manipulations of nanoscale materials that result in physical-chemical properties changes or other defining characteristics (e.g., surface modifications of nanotubes to enhance solvent dispersibility)?*
- *Question 8: How should physical-chemical property data be prioritized for the NMSP? Based on availability, effect (toxicity or exposure criteria), or other factors?*

Discussion: Nanoscale Manufacture and Processing
- *Question 9: What are the common processes used to manufacture nanoscale materials?*
- *Question 10: How are processes used to produce specific characteristics or properties?*
- *Question 11: Which methods reduce particle size but do not result in property changes? Which methods reduce particle size and result in property changes?*

Noon   LUNCH

1:15 p.m.   **Discussion: Methodologies for Characterizing Nanoscale Materials**
      Analytical Methods:
- *Question 14: Are validated methods available for the different categories of nanoscale materials?*
- *Question 15: Are there techniques that can be universally applied?*
- *Question 16: For small quantities of materials, are there sampling, handling, and collection techniques as well as sample integrity, accuracy and precision QA/QC methodologies available?*
- *Question 17: What is the status of standardization efforts? Are these efforts focused on broadly applicable characterization methods or category-specific methods?*
- *Question 18: What alternative or innovative methods or technologies can be applied to nanomaterial analysis?*
      Models:
- *Question 19: Are there models that are currently used to obtain property data for nanoscale materials? For which properties and which nanoscale materials?*
- *Question 20: Has any validation work been conducted that compares predicted values with measured data? For which properties and which nanoscale materials?*
- *Question 21: Are there current significant characterization needs for which the NMSP should investigate model development?*

Discussion: Prioritization of Data Gaps

2:30 p.m.   BREAK

2:45 pm   Public Comments

3:15 p.m.   Panelist Closing Remarks

3:45 p.m.   Next Steps and EPA Closing Remarks

4:00 p.m.   ADJOURN

Appendix B

DISCUSSION PAPER

Peer Consultation Public Meeting on
The Material Characterization of Nanoscale
Materials

Discussion Topics

August 7, 2007

Peer Consultation Public Meeting on
The Material Characterization of Nanoscale Materials
Discussion Topics

Meeting Background and Purpose

EPA is convening a public scientific peer consultation meeting on material characterization for nanoscale chemical substances ("nanoscale materials") to inform the development of its Nanoscale Materials Stewardship Program (NMSP) under the Toxic Substances Control Act (TSCA). The peer consultation is one of several actions EPA is taking to better understand the potential risks and benefits of nanotechnology.

On October, 18, 2006, EPA invited the public, industry, environmental groups, other federal agencies and other stakeholders to participate in the design, development and implementation of a stewardship program for nanoscale materials. On July 12, 2007, EPA announced the availability of a NMSP concept paper and related documents and a public meeting on August 2, 2007 to discuss and receive comments on these materials (http://www.epa.gov/oppt/nano/nmspfr.htm).

The NMSP will complement and support the Agency's new and existing chemical programs under TSCA and will help provide a firmer scientific foundation for regulatory decisions by encouraging the development of key scientific information and appropriate risk management practices for new and existing chemical nanoscale materials. The NMSP is intended to include but not limited to engineered nanoscale materials manufactured or imported for commercial purposes as defined in 40 CFR 720.3 (r). The NMSP is envisioned to have two parts: 1) a Basic Reporting Program and 2) an In-depth Program. This discussion paper and the public peer consultation meeting are intended to support the NMSP by identifying material characterization data that participants could submit under the Basic Program if they are in the participant's possession or are reasonably ascertainable as defined in 40 CFR 720.3 (p). The data and experience generated by the basic reporting phase will help to inform the types of in-depth data to be developed. In-depth data development could begin at any time and would entail, among other types of data, development of material characterization data in a greater amount of detail. In-depth data development could also include additional types of material characterization data if they are identified.

EPA received input in November, 2005 from the National Pollution Prevention and Toxics Advisory Committee (NPPTAC) regarding a voluntary stewardship program for nanoscale materials. A NPPTAC ad-hoc work group on nanoscale materials developed an overview paper that stated that a voluntary stewardship program should:

1. Give EPA, and the public to the extent possible recognizing legitimate CBI issues, a better understanding of the types of engineered nanoscale materials; the physical, chemical, hazard and exposure characteristics of such substances; the volume of such substances; and the uses of such substances;

2. Help EPA develop capacity and a process to identify and assess risks of engineered nanoscale materials;

3. Help EPA determine what information it needs about engineered nanoscale materials and articulate those information needs to industry and other stakeholder groups;

4. Help EPA understand what risk management practices are being used at production, processing, use and disposal stages, and what additional risk management practices need to be implemented;

5. Prompt or reinforce the implementation of risk management practices; and

6. Provide the information and experience needed to develop an overall approach to the treatment of nanoscale chemical substances under TSCA that builds public trust in nanoscale materials while enabling innovation and responsible development.

EPA will utilize all public input, including that from NPPTAC, other stakeholders, public meetings and peer consultations to further inform the development of its Nanomaterials Stewardship Program and TSCA program for nanoscale materials.

Meeting Objectives

The EPA public peer consultation meeting on material characterization needs for nanoscale materials will help clarify which data and elements should be included in the NMSP Basic Program and/or In-depth Program. The goal is to have an applied discussion that considers 1) the currently available understanding of material characterization as it relates to nanoscale materials and 2) how this understanding can be used to guide the Agency's thinking regarding the material characterization data elements that would be most useful and important to include in the NMSP. The specific objectives of the public peer consultation meeting are as follows:

1. To inform industry and the public of EPA's level of understanding of the material characterization needs for nanoscale materials in general and for the NMSP;

2. To further develop EPA's understanding of how nanoscale materials are engineered or manufactured to achieve specific properties and characteristics;

3. To further develop EPA's understanding of which chemical identification elements and physical-chemical property data are generally relevant in characterizing nanoscale materials and which identification elements and property data are most important in characterizing specific classes of nanoscale materials;

4. To discuss what analytical procedures and test methods are available for acquiring these material characterization data, and where procedure and method validation or development is needed;

5. To discuss how these material characterization data needs should be prioritized for the NMSP Basic and/or In-depth Program; and

6. To discuss potential nomenclature needs for specific classes of nanoscale materials based on material characterization outcomes.

Discussion Overview

Despite the rapid advancement of nanotechnology, the breadth of nanoscale material types coupled with the limited hazard data available for many of these materials pose a challenge in understanding and measuring their benefits and risks. Numerous efforts are underway to begin to address these challenges. The International Life Sciences Institute (ILSI) Research Foundation/Risk Science Institute, for example, convened an expert working group to develop a strategy for identifying hazards associated with engineered nanoscale materials. Focusing on the limited data available, the working group developed a screening strategy for hazard identification (rather than a detailed testing protocol) that includes a broad data gathering effort.[1]

The ILSI report describes the characterization of nanoscale materials, in addition to in vitro and in vivo screening, as a key third aspect of an overall screening strategy due to the likely dependence of the biological activity of nanoscale materials on physical-chemical properties not often considered in toxicity screening studies. Additionally, given the difficulties associated with characterizing many nanoscale materials, nanoscale material characterization is a subject appropriate for detailed investigation and discussion.

Numerous national and international standards organizations, including the American National Standards Institute (ANSI)[2] and the International Organization for Standardization (ISO)[3], have also convened committees to begin to address many of these same challenges and in particular the need for methods standardization. Several of these committees have indicated that, given the breadth of nanotechnology and its data issues, the initial products the committees develop may be in the form of best practices rather than actual test protocols.

The remainder of this paper discusses a proposed approach for the EPA scientific peer consultation meeting on nanoscale material characterization. EPA recognizes that the different chemical classes of nanoscale materials would make universal application of any particular characterization endpoint or methodology impossible. The premise of the Basic Reporting phase of the NMSP is that some information is known or reasonably ascertainable. The approach for this Panel is therefore to discuss what data are known or reasonably ascertainable to characterize nanoscale materials. This will be followed by a discussion on the methodology used to obtain and use characterization endpoints of interest.

Discussion Topics

I. Characterization of Nanoscale Materials

1. Description of nanoscale materials
   a. Types/categories of nanoscale materials

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[1] http://www.particleand fibretoxicology.com/content/2/1/8
[2] http://www.ansi.org/standards_activities/standards_boards_panels/nsp/overview.aspx?menuid=3
[3] http://www.iso.org/iso/en/stdsdevelopment/tc/tclist/TechnicalCommitteeDetailPage.TechnicalCommitteeDetail?
COMMID=5932

2. Physical-chemical properties of potential interest
    a. Particle size and distribution
    b. Particle shape and dimensions
    c. Agglomeration and aggregation
    d. Surface area
    e. Surface charge
    f. Surface chemistry
    g. Chemical composition
    h. Crystal structure
    i. Impurity identification and levels

3. Design to achieve unique properties
    a. Manufacturing and processing methodologies
    b. Chemical transformations

II. Methodologies for Characterizing Nanoscale Materials

1. Obtaining characterization data for nanoscale materials
    a. Analytical methods for detecting and quantifying nanoscale materials
    b. Analytical methods measuring physical-chemical properties (measurement techniques and testing protocols)
    c. Models to predict properties and effects

2. Metrology
    a. Methods validation
    b. Standards and harmonization

3. Prioritization of characterization data and data gaps

4. Miscellaneous – Do Panelists have additional topics to discuss?

Discussion

This section provides additional information on specific technical issues to facilitate discussion at the meeting. The information will include literature findings as well as questions on the specific discussion topics.

Types of Nanoscale Materials & Their Structures and Chemical Compositions

Based on structure and chemical composition, EPA has grouped nanomaterials into 4 distinct categories for purposes of this discussion: 1) simple organic molecules; 2) simple inorganic molecules; 3) polymeric substance (including dendrimeric substances); and 4) composites. A fifth category, biological compounds will not be addressed in the peer consultation. While all of these categories can be divided further, only the organic category will be divided further into molecules based predominantly on carbon (e.g., fullerenes, nanotubes) and all other organic substances (e.g., salts of carboxylic acids). This grouping is similar to the American National Standards Institute Nanotechnology Standards Panel approach presented at

the September 2004 meeting at the National Institute of Standards and Technology in Gaithersburg Maryland[4].

Question 1: Are there any other significant categories, based on structure and chemical composition, that should be included in this discussion because they are substantially different from the categories mentioned (e.g., hybrids, self- assembly devices, others)?

Question 2: For the different categories of nanoscale materials, what is the current state of knowledge about structure and chemical composition?

Question 3: Can structures and chemical composition be correlated to specific properties and is this correlation quantifiable?

Physical-Chemical Properties

The importance of nanoscale materials is due to their potential for unique or greatly enhanced properties. EPA routinely uses a base set of physical-chemical property data (e.g., melting point, boiling point, vapor pressure, water solubility) for a variety of programs (e.g., High Production Volume Challenge, New Chemicals) and decision-making. As mentioned previously, certain material properties are of significant importance in characterizing nanoscale materials. Recent research suggests that particle size, surface area, and surface chemistry (or surface activity) are initially some of the most important properties to measure.

As expected for most chemicals, class 1[5] substances having specific molecular structures and formulas may be more readily studied and characterized at the nanoscale than the polymer and composite categories. For example, carbon-based nanoscale materials as well as metal oxide nanoscale chemicals are often well characterized. Structural and physical-chemical property data therefore are likely to be well documented for these types of materials.

Question 4: Which physical-chemical properties are relevant to nanoscale materials and how? Which are known or reasonably ascertainable and which have data gaps?

Question 5: Are there properties that would have little or no relevance under the NMSP?

Question 6: Which properties are associated with aggregated or agglomerated nanoscale materials, as opposed to properties that are inherent to the material regardless of physical form?

Question 7: Are there routine manipulations of nanoscale materials that result in physical-chemical properties changes or other defining characteristics (e.g., surface modifications of nanotubes to enhance solvent dispersibility)?

Question 8: How should physical-chemical property data be prioritized for the NMSP? Based on availability, effect (toxicity or exposure criteria), or other factors?

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[4] http://publicaa.ansi.org/sites/apdl/Documents/Forms/AllItems.aspx
[5] Class 1 substances are distinct chemicals with known, non-variable molecular structures

Nanoscale manufacturing and processing

The number of manufacturing and processing methods for generating nanoscale material continues to grow and become more sophisticated. The two primary areas for this discussion include physical reduction methods (milling) and engineering methods (e.g., particle stabilization, vapor deposition, self assembly).

Question 9: What are the common processes used to manufacture nanoscale materials?

Question 10: How are processes used to produce specific characteristics or properties?

Question 11: Which methods reduce particle size but do not result in property changes? Which methods reduce particle size and result in property changes?

Impurities

Impurity content is a growing area of interest in nanotechnology due to improved performance observed in some cases (e.g., solar cells and semiconductors) and deleterious effects observed in others (e.g., quantum dot quantum computers). The confounding effects that impurities have with respect to toxicological endpoints are also being studied (National Nanotechnology Initiative 2006 Environmental Health and Safety research report[6]).

Question 12: How important are impurity identity and impurity levels to the understanding and characterization of nanoscale materials?

Question 13: Are there routine purification procedures that can effectively control or remove impurities, when desirable, for certain classes of nanoscale materials?

Obtaining characterization data

Determining identity, quantifying the nanoscale particle range, and measuring physical-chemical properties for that identity and particle range are essential to the characterization of nanoscale materials. Because of the challenges associated with size, shape, surface characteristics, and possibly other aspects of nanoscale materials, an evaluation of existing measurement techniques is critical to nanoscale material characterization. The National Nanotechnology Initiative report stated that "...Accurate and useful measurement techniques are also important because agglomerated nano materials may either retain or lose their emergent properties - or take on new properties - thus affecting the potential biological response."

Question 14: Are validated methods available for the different categories of nanoscale materials?

Question 15: Are there techniques that can be universally applied?

Question 16: For small quantities of materials, are there sampling, handling, and collection techniques as well as sample integrity, accuracy and precision QA/QC methodologies available?

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[6] http://www.nano.gov/NNI_EHS_research_needs.pdf

<u>Question 17</u>: What is the status of standardization efforts? Are these efforts focused on broadly applicable characterization methods or category-specific methods?

<u>Question 18</u>: What alternative or innovative methods or technologies can be applied to nanomaterial analysis?

Modeling

Empirical modeling can be a useful approach to predict physical-chemical properties when experimental data are not known or ascertainable. The initial problem with modeling is that, to accurately predict property endpoints for a given category of substances, there must be some experimental data available in the tool's database for at least some representative substances in that category. For newly discovered or studied materials, the minimum but necessary quantity and type of experimental data often is not available to sufficiently populate a tool's database and allow accurate prediction by the tool. Some estimation methods have been developed for specific property endpoints, but many others are lacking.

<u>Question 19</u>: Are there models that are currently used to obtain property data for nanoscale materials? For which properties and which nanoscale materials?

<u>Question 20</u>: Has any validation work been conducted that compares predicted values with measured data? For which properties and which nanoscale materials?

<u>Question 21</u>: Are there current significant characterization needs for which the NMSP should investigate model development?

Appendix C

PRELIMINARY PANEL COMMENTS ON DISCUSSION PAPER

Comment Compilation of Panelists' Preliminary Responses to Panel Discussion Questions Posed in the Discussion Paper for the Public Meeting on Material Characterization of Nanoscale Materials

Draft Compilation as of August 31, 2007

A compilation of Panelists' preliminary comments received in response to "Panel Discussion Questions" contained in the Discussion Paper for the Public Meeting on Material Characterization of Nanoscale Materials is provided below. This compilation is organized as follows:

a) General Observations
b) Discussion Category Responses
      a. Types of Nanoscale Materials and their Structures and Chemical Compositions
      b. Physical-Chemical Properties
      c. Nanoscale Manufacturing and Processing
      d. Impurities
      e. Obtaining Characterization Data
      f. Modeling
c) Supporting Figures
d) Supporting References

General Observations

Dr. Warheit:

Thank you for giving me the opportunity to provide some preliminary comments on the Discussion Topics for EPA's Peer Consultation Public Meeting on the Material Characterization of Nanoscale Materials. The forthcoming meeting should provide useful information, both for EPA and for stakeholders/outside participants. I would caution EPA, that in my opinion, many of the answers to the questions contained in the "Discussion Topics" document are case-by-case and/or nanoparticle-type specific (e.g. those pertaining to metal oxides vs. carbon structures) and a universal response cannot be given (e.g. questions 9 and 10 – "What are the common processes used to manufacture nanoscale materials? And "How are processes used to produce specific characteristics or properties"). Indeed the various subsets of nanoparticle-types are likely to require different answers to the same questions.

Discussion Category Responses

Types of Nanoscale Materials & Their Structures and Chemical Compositions

Based on structure and chemical composition, EPA has grouped nanomaterials into 4 distinct categories for purposes of this discussion: 1) simple organic molecules; 2) simple inorganic molecules; 3) polymeric substance (including dendrimeric substances); and 4) composites. A fifth category, biological compounds will not be addressed in the peer consultation. While all of these categories can be divided further, only the organic category will be divided further into molecules based predominantly on carbon (e.g., fullerenes, nanotubes) and all other organic substances (e.g., salts of carboxylic acids). This grouping is similar to the American National Standards Institute Nanotechnology Standards Panel approach presented at the September 2004 meeting at the National Institute of Standards and Technology in Gaithersburg Maryland[7].

Question 1: *Are there any other significant categories, based on structure and chemical composition, that should be included in this discussion because they are substantially different from the categories mentioned (e.g., hybrids, self- assembly devices, others)?*

Dr. BelBruno:

The classification scheme presented seems to be the most practical. To do otherwise, would overwhelm the issue at a time when we are trying to get a basic set of data together. Most other nanoparticles are combinations or composites of the classes already listed, for example, polymer coated iron nanoparticles for imaging. While the combined material will need to (eventually) be examined for its own possible unique properties, the components need to be understood first.

Self-assembled and hybrid materials, cited in the topic description, fall into the same composite or "combination" category as the imaging particles noted above.

Dr. Canady:

Perhaps the categories, or EPA's thinking about "mechanisms to help clarity of approach," should not be limited by structure AND composition. A useful classification scheme (including cross-referenced sub-classifications across categories) should probably be more amenable to identifying attributes that are important to regulatory clarity by being open to structure, composition, or combinations of structure and composition. From asbestos and PM data it seems there may be combinations of structure and composition that are important and there may also be structures that are important independent (to a large degree) of composition. Function (e.g., OP compounds, endocrine active compounds) may also be a useful classification attribute. Classification should be amenable to a matrix approach with composition, structure, and function as dimensions of the matrix. It seems likely, as more and more NMs are developed, that the very broad categories presented here by EPA will begin having very little practical utility because we will be discovering categories and classes within them that are more important in understanding how to regulate, and that confuse the boundaries.

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[7] http://publicaa.ansi.org/sites/apdl/Documents/Forms/AllItems.aspx

If EPA stays with large bins, here are some other categories to consider:

Combinations: There are already intersections of inorganic/organic/polymer/composite materials in the form of specifically constructed nanoscale materials or particles that will challenge any category-based approach to nomenclature or regulation. At some point the combinations may comprise the bulk of the applications as molecular assembly comes into full use.

Emulsions, vesicles, liposomes, micelles: This would be a structure (or form) rather than composition category with either amphiphillic molecules forming "filled balloons" or small collections of molecules forming nanoscale particles in stasis in a liquid. These kinds of particles are being developed for use in food and drug applications, biocides, and, most likely, pesticides. Based on the utility of the approach in drug delivery and nutrient stabilization in foods, it's not hard to imagine that industrial uses would also be developed (for chemical processes to control/specify reactions, solubilization of insoluble intermediates, and transport/stabilization of compounds).

Dr. Cassell:

I'm confused at this terminology defined in the introductory paragraph. For instance, simple organic molecules would not be inclusive of multiwalled carbon nanotubes (this is implied that it can be done this way). I think there needs to be more thought put into classifying these materials correctly.

Hybrid structures in particular need to be considered. For example, there are emerging reports detailing the use of engineered nanomaterials that could be placed into multiple categories (organic, inorganic and polymeric). Filled carbon nanostructures (e.g. metal filled multiwalled carbon nanotubes) are a class of materials that could be grouped in such a way. Another common class of materials is hybrid inorganic/organic polymers.

Dr. Lahann:

In principle, the four categories are sufficient for broad classification on a materials basis. However, hybrid materials have also been observed increasingly in recent times and are distinct from composites. For instance, a particle with an inorganic core and an organic shell should be considered a hybrid material rather than a composite. Thus, adding a 5th category may be wise.

Dr. Murashov:

The concept paper proposes the following grouping of nanomaterials: 1) simple organic molecules; 2) simple inorganic molecules; 3) polymeric substances (including dendrimeric substances); 4) composites; 5) biological (nanomaterials assigned to the latter group are not considered under the proposed stewardship program). The first category is further subdivided into molecules based predominantly on carbon (e.g. fullerenes and nanotubes) and all other organic substances (e.g. salts of carboxylic acids).

This categorization suffers from inconsistency with the conventional usage of chemical terms. Specifically:

   a) Carbonaceous materials including allotropes of carbon such as fullerenes and carbon nanotubes are considered inorganic materials.
   b) It is not clear why nanoscale formulations of simple organic molecules such as salts of carboxylic acid were made into a separate category of nanomaterials given their little significance as engineered nanomaterials at this point.
   c) Traditionally, "simple inorganic molecules" means molecules such as CO_2, SO_2, NO, H_2O. It is not clear why nanoscale formulations of these materials would be made into a separate category of nanomaterials given their little significance as engineered nanomaterials at this point. Was this category meant to describe "simple inorganic compounds" to include solid materials with a high degree of ionic or metallic bonding such as TiO_2 and Ag, respectively?
   d) It is not clear what is meant by "composite" category of nanomaterials. For example, would nanoparticles composed of cores and shells of different chemistries be considered composites? Would functionalized nanoparticles be considered composites?

An alternative classification of simple nanomaterials can be found, for example, in the draft ISO TC229 Technical Report on "Health and Safety Practices in Occupational Settings Relevant to Nanotechnologies" and includes the following categories: 1) carbonaceous; 2) oxides; 3) metals; 4) semiconductors; 5) organic polymeric; 6) bio-inspired. This classification is better aligned with the chemistry of simple nanomaterials and their hazard properties. More complex nanomaterials could be categorized further along the lines of nomenclature tree being developed by ISO TC229 which includes distinction between nano-objects (includes nanoparticles) and nanostructured materials (includes nanocrystals and complex assemblies of nano-objects).

Dr. Murday:

   a) The presently identified categories apparently presume freestanding nanostructures (NS). These certainly pose a greater risk for pervasive dissemination. One might also think about NS embedded physically in a matrix (i.e. no chemical bonding), embedded chemically in a matrix (i.e. chemical bonding) and what might be called "thin film" where NS are etched out of deposited films (that are chemically bonded to the substrate).
   b) There is some danger in working from a molecular perspective of NS. Molecules are largely well defined entities – one knows exactly what atoms are present and their first order bonding structure (some second order effects like chain folding, cis/trans folding might not be known). Nanostructures will almost never be that precisely known – if for no other reason than the number of atoms can be too large for effective, specific nomenclature.
   c) There is benefit from utilizing a molecular perspective. There is policy/procedure in place to handle hazardous molecules. That policy/procedure should be adequate (albeit over constraining) to handle NS safely. NS are not some brand new form of matter that presents a totally new set of problems.

Dr. Small:

   a) EPA should probably include a category for "elemental" nanoparticles, i.e. Au, Ag, Cu,

or are these considered simple inorganic molecules. To me they seem to be a separate class from say oxides like TiO2.
  b) Which category includes layered nanomaterials?
  c) Is EPA considering only those materials with 3 nanodimensions i.e. particles?
  d) Where do functionalized surfaces fit in this scheme?

Dr. Warheit:

No specific comments provided.

Question 2: *For the different categories of nanoscale materials, what is the current state of knowledge about structure and chemical composition?*

Dr. BelBruno:

Pure materials (perhaps better defined as single component?) can be well-defined. For example, pure carbon-based materials such as fullerenes or nanotubes may be well-characterized by size, microscopy and chemical functionality. Generally impurities consist of remnants of metal catalysts, which can be detected and (usually) removed. Similarly, inorganic materials such as TiO_2 nanoparticles may be characterized by size and structure using standard techniques (described elsewhere in response to subsequent questions).

Polymer nanoparticles can present problems with respect to chemical composition, but may be characterized by size distribution using standard methods. Chemical composition is more difficult since, even for macroscopic polymers, there are impurities and mixed compositions. This problem can probably be solved, but requires more attention. Composite materials fall into this same category.

Dr. Canady:

I don't have this detailed knowledge but based on the breadth of the 4 categories, not sure the question is answerable. If the question were about carbon nanotubes versus quantum dots or dendrimers you could start forming a basis for the state of knowledge, but where do you start and end in a description of the state of knowledge about particles of inorganic materials?

Dr. Cassell:

For newer nanomaterials systems, knowledge concerning structure and composition varies widely. Some nanomaterials systems are very well characterized whereas in other systems, I would say there is little if any knowledge. The extent to which characterization is performed is often determined by the particular study involved and whether the researchers/users of the material required this additional characterization for understanding their system.

Dr. Lahann:

For categories 1 and 2, chemical particle compositions and often also structures are in many cases well understood. Mostly, the role of impurities is often hard to assess and their influence on structure maybe underestimated in cases. How composition and structure relates to function is however often unclear and more work will need to be done. Nanomaterials in categories 3 and 4 are often less well characterized. Synthetic polymers are associated with variations of their molecular weight and structure, which equally holds for nanomaterials comprised of polymers. The extent of this variability may change between different polymer materials, but is always present at least to some extend. Again, it may be important to assess the role of this variability with respect to function. Similarly, composite materials can be associated with substantial variability in chemical composition and structure.

Dr. Murashov:

It is possible to characterize relatively well almost any nanoscale material. The main challenge is that this characterization is time consuming, expensive and limited to only a small sample. At the present state of the development, many nanomaterials have wide distributions of chemistries and structures within the same product batch. This heterogeneity of chemical composition and structure results in a range of properties which are not fully characterized by conventional measurements.

Dr. Murday:

   a) Presumably NMSP is presently focusing on free standing nanostructures.
   b) In the chemistry/physics research laboratories, where there is generally careful attention to the identity of the NS being measured, the state of knowledge is okay. But there remain limitations to the analytical tools available for full characterization of NS (as is true generally for all materials).
   c) There is generally poor knowledge from the standpoint of large scale NS batches where separation/purification problems are more difficult/expensive to handle. This problem is clearly illustrated in carbon nanotubes.   A couple of years ago one researcher purchased CNT from two vendors and multiple batches. He carefully analyzed the procured material. See Figure A. The worst batch had only 40% carbon in it – and not all of that carbon was likely to be CNT.
   d) NS will frequently (usually) have "surface treatments" or "shells" to make them compatible with exposure to their intended environment. In the case of medical/health applications, the shell will likely impart recognition/transport functions. Those same two functions clearly have environmental implications.
   e) Another level of challenge is the role of "interphase" – roughly speaking a layer of material enveloping/separating the NS.

Dr. Small:

Simple organic molecules: The structure and chemical composition of the fullerenes and C nanotubes are well known for the specific nanomaterials. However when these materials are sold

as a product the product can be very inhomogeneous and the purity of the commercial material (structure and chemical composition) is often unknown.

Simple inorganic molecules: The structure and chemical composition of simple inorganic molecules like salts and metal oxides are for the most part well known. The purity of the commercial product is more homogeneous that commercial nanotubes but can still be an issue depending on how the nanoproduct is made.

Polymeric materials: For materials like dendrimers, lipids the theoretical structures are known to the degree this structure applies to what is sold is unknown i.e. particularly for functionalized systems % coverage. For polymeric materials that "contain" specific compounds there is very little validation of structure.

Composites: For more complex materials multifunctional, layered, phase separated etc. the current state of structure and chemical composition is much less well known if at all. Several initial studies of complex nanomaterials indicate that there is significant variation with respect to structure and composition.

Dr. Warheit:

No specific comments provided.

Question 3: *Can structures and chemical composition be correlated to specific properties and is this correlation quantifiable?*

Dr. BelBruno:

As a general rule, we are not at the point where we can correlate physical properties to chemical structure/composition. The properties have the potential to be size-dependent and until theory can provide a rigorous method to describe the change in properties with size, this desirable goal cannot be claimed. For example, silver nanoparticles, in a select size range, may have antibacterial activity (C. N, Lok, et al, *Journal of Biological Inorganic Chemistry* (2007), **12**, 527), but this is not a prior, predictable. Magnetic properties of nanoparticles also show a size and structure dependence (S Kar, et al, *Journal of Nanoscience and Nanotechnology* (2006), **6**, 771; Y.T. Jeon, et al, *International Journal of Modern Physics B* (2006), **20**, 4390).

Dr. Canady:

Yes and yes. Why is this considered even potentially uncertain?

Dr. Cassell:

Yes to a certain extent. For instance, we know that surface area of nanoscale materials increases as diameter of the material decreases. However, direct correlation between chemical

composition and reactivity for instance is not as well understood for nanoscale systems. I do think it is quantifiable in a qualitative sense, but perhaps not in a quantitative sense.

Dr. Lahann:

Structure and chemical composition are critical aspects of every nanomaterial, and as such contribute to the specific properties of a material. In some cases, there is a direct correlation to a specific property, which then is quantifiable. In other cases, structure and composition influence specific properties through secondary mechanisms. For instance, nanomaterials of category 3 may be water-soluble and as such highly resorbable, unless the polymer chains are further crosslinked. The extent of crosslinking may directly correlate with the solubility of a material and its resorption. Similarly, molecular weight or chirality of polymers can influence resorption and degradation kinetics significantly.

Dr. Murashov:

Yes. Structure and chemical composition can be quantifiably correlated to specific risk-related properties. Examples are:
   a) Aerodynamic properties as a function of particle aerodynamic diameter (Lee and Liu, 1982);
   b) Surface area as a dose metric for low solubility low toxicity particulate matter (Maynard and Kuempel, 2005);
   c) Biological response as a function of surface charge (e. g. for dendrimers see Jevprasesphant et al, 2003; Mecke et al, 2004);
   d) Biological response and pharmacokinetics as a function of surface chemistry (e. g. for functionalized fullerenes and carbon nanotubes see Sayes, 2004; Singh et al, 2006).
Once nanomaterial's structure approaches atomic-level consistency (nano-molecular materials?), it should be straightforward to develop and reliably employ Quantitative Structure Activity Relationship - like models. Presently, we have very limited number of nanomaterials which have such an atomic-level consistency. An example of nanomaterials where we do have such atomic level control over chemical structure is dendrimers.

Dr. Murday:

   a) As an academic question, the answer is definitively yes – composition/ structure will define the chemical/physical properties – on NS as well as any other form of material. As a practical question the correlation is difficult because samples are generally not the same level of purity as we expect for chemical reagents.
   b) I have included a preliminary table that identifies selected properties and "critical scale lengths," i.e., properties that will likely vary significantly from predictions of the solid state (larger scale) / chemical (smaller scale) models we presently use. See Figure B. There could be real value for EPA to sanction a careful examination of "critical scale lengths" to identify when problems would be more likely to occur.
   c) One area that is very important is chemical reactivity – clearly an environmental issue. Surface science has developed reasonable understanding of reactions on atomically flat surfaces, and some understanding of vicinal surfaces (where atomic scale "roughness" is

higher). But NS have surfaces more complex than these. The research on catalysis does address some of that complexity, but the understanding - because of the difficulty in preparing well defined samples, characterizing them, and modeling the higher level of complexity – is not as good.

Dr. Small:

For some properties the answer is yes, for example the color or absorption of light by specific sized Au particles. The correlation for most properties related to risk assessment and EHS is not well known. Efforts to understand these types of correlation are hindered by lack of validated "standards" and methods.

Dr. Warheit:

No specific comments provided.

Physical-Chemical Properties

The importance of nanoscale materials is due to their potential for unique or greatly enhanced properties. EPA routinely uses a base set of physical-chemical property data (e.g., melting point, boiling point, vapor pressure, water solubility) for a variety of programs (e.g., High Production Volume Challenge, New Chemicals) and decision-making. As mentioned previously, certain material properties are of significant importance in characterizing nanoscale materials. Recent research suggests that particle size, surface area, and surface chemistry (or surface activity) are initially some of the most important properties to measure.

As expected for most chemicals, class 1[8] substances having specific molecular structures and formulas may be more readily studied and characterized at the nanoscale than the polymer and composite categories. For example, carbon-based nanoscale materials as well as metal oxide nanoscale chemicals are often well characterized. Structural and physical-chemical property data therefore are likely to be well documented for these types of materials.

Question 4: *Which physical-chemical properties are relevant to nanoscale materials and how? Which are known or reasonably ascertainable and which have data gaps?*

Dr. BelBruno:

The prelude to the questions really has it all there. The important properties (aside from chemical structure/composition) are particle size, surface area and reactivity. Size, surface area and structure are measurable and standard procedures are possible, but reactivity is more difficult to quantify and often particle specific. Techniques such as dynamic light scattering, BET procedure and x-ray diffraction, respectively, are routinely employed.

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[8] Class 1 substances are distinct chemicals with known, non-variable molecular structures

Dr. Canady:

It seems you have to first express the decisions that need to be made about the materials and then what is minimally sufficient for decisions and therefore relevant. I am afraid I am not familiar enough with TSCA to begin this process.

There is a risk here of developing lists of what we can do or can think of and then letting them stand forever as requirements. The list of relevant properties should flow from the decision needs. For example, some recent reviews focus on surface area because of the thought that it may correlate better with toxicity than mass does. However, other measures may be enough to capture the listing decision needs or even reportable quantity determinations without the need to measure surface area in any specific way.

Basing property needs on a minimally sufficient set to make decisions could more easily consider both cost and utility so that unnecessary costs can be avoided.

On the other hand, at least initially and until we get a better sense of what is useful for decisions, the response to this question could be rather long for the general case of all nanoscale material so that we can begin to sort through the utility functions.

There are likely to be properties relevant to decisions about specific classes of materials but not others.

Dr. Cassell:

The most relevant physical-chemical properties are crystallinity, surface properties (charge, roughness, and area), and solubility state of agglomeration because these properties directly influence the direct assessment of toxicological risk. I think that most new materials the crystallinity has been characterized well but the state of agglomeration is probably not as well understood.

Dr. Lahann:

As for all other materials, nanoscale materials have specific sets of properties that can be used for characterization. In addition, these materials often have specific properties due to the nanoscale information encoded in their structure. This information may be transient and alter with temperature. For instance, for a given CdSe nanoparticle material, its optical properties are – within a certain range – a function of the particle diameters. Another example is the magnetic properties of iron oxide particles, which alter with diameter. In general, properties that are associated with the nanoscale structure of a material are better suited for characterization of nanomaterials. Often, the characterization of individual nanoobjects on the basis of size and size distribution, shape and shape distribution, chemical composition, structure, etc., may be more insightful than "bulk characterization".

Dr. Murashov:

All listed physical-chemical properties in the discussion paper are relevant to risk potential of nanomaterials:
   a) Particle size and distribution
   b) Particle shape and dimensions
   c) Agglomeration and aggregation
   d) Surface area
   e) Surface charge
   f) Surface chemistry
   g) Chemical composition
   h) Crystal structure
   i) Impurity identification and levels
In addition, the following are important parameters for characterization (see also draft ISO TC229 TR "Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment"):
   1) Solubility;
   2) Porosity;
   3) Stability under different experimental conditions (biopersistence, durability and dispersibility).
In particular, since interactions between biological species and nanomaterials occur primarily at the nanomaterial surface, it is critical to characterize not just the chemistry of nanomaterial core, but also chemical species represented on the surface of nanomaterials. For example, if one considers titania nanoparticles coated with silica and made to reflect UV light, the commercially important active ingredient is titania. However, since silica is on the surface, the biological response is expected to be to silica at least initially until silica coating is dissolved or otherwise altered.

All properties can be measured at a research level. However, presently atomic-level description is limited to individual nanoscale features such as individual nanoparticles. There is a lack of standardized methods and techniques to conduct characterization of representative nanomaterial samples routinely that is cost- and time-effective.

Dr. Murday:

   a) All physical/chemical properties are relevant to NS. Perhaps the more important question is which of those properties might vary significantly at the nanoscale and are those properties of environmental concern.
   b) For the NS itself one can address this question by examining property vice critical scale length – two generic specific issues to watch for are quantization (size) and correlation (many atoms) effects.
   c) Many, if not most NS, will have a core/shell(s) structure – i.e., they will not be a monolithic entity. The shell(s) will impart different/additional physical and chemical properties. As we move more to "smart" structures – those that sense and respond - the complexity of those added properties will grow.

d) In addition, NS can have an "interphase." This will be a tougher problem since the "interphase" may be generally even more complex and hard to characterize.

Dr. Small:

I think it will be important to define relevance to what. In general, properties such as size, surface area, chemical structure, surface characteristics, functionalization, and charge are relevant to the properties of most nanoscale materials. Different materials will have a different set of properties that are relevant to its use or hazard evaluation. Most methods for the determination of physical-chemical properties of nanomaterials have large gaps. Critical to minimizing these gaps is to provide information that is consistent and reproducible from one lab to the next. Accuracy may well be a secondary consideration.

Dr. Warheit:

No specific comments provided.

Question 5: *Are there properties that would have little or no relevance under the NMSP?*

Dr. BelBruno:

At this point, it is not at all clear which, if any, properties have little relevance. I do not believe that any measurements should be ignored or discouraged by the standards that might be set.

Dr. Canady:

There is probably a list of things that are not relevant from the chemical realm, which EPA might have included in a list of what to ask for out of habit. For example, volatility measures that you might require for chemicals may be valueless.

I can't think of a case for a categorical exclusion on a property other than things like volatility (i.e., property measures that just come up blank because they don't apply to particles). We probably don't know what will have little or no relevance in all cases. There would need to be a theoretical basis to "prove a negative" and this may be particularly difficult while we are in the middle of discovering behaviors and uses as a result of manipulating properties.

Perhaps when SAR or other methods of relating properties to function evolve to a point of understanding null points in chemical and biological interaction we can begin to develop maps of where there are not effects. Seems likely they would be multivariate likelihood statements rather than "this property never interacts" with biology.

Dr. Cassell:

I think all of the information is useful. In certain contexts, optical properties (photoelectric properties for instance) would not be relevant to NMSP. All of the well-accepted physical chemical properties should be relevant to NMSP however.

Dr. Lahann:

See response to question 4.

Dr. Murashov:

At this point, it appears that the following properties have little direct relevance to the risk potential of nanomaterials:
   a) thermal properties such as thermal conductivity, heat capacity and thermal diffusivity;
   b) optical properties such as refractive index, optical rotation, luminescence;
   c) elastic properties such as Young modulus and elastic constants.

Dr. Murday:

   a) Presumably the emphasis is on impacting living systems. There is not enough NS volume to worry about unsightly/unwieldy waste piles.
   b) Some likely properties with little/no relevance from an environmental protection perspective - thermal conductivity, plasmonics, superconductivity.

Dr. Small:

It is reasonable to assume that so little is known regarding the relationship between properties and impacts that most properties have to be considered relevant until definitive studies are conducted to show they are not relevant. That said, I would think the determination of mass alone will not be relevant to NMSP.

Dr. Warheit:

No specific comments provided.

Question 6: *Which properties are associated with aggregated or agglomerated nanoscale materials, as opposed to properties that are inherent to the material regardless of physical form?*

Dr. BelBruno:

If we are dealing with aggregates or agglomerated materials, we need to measure those properties cited above, size, surface area, reactivity) for both the components and the aggregates. Essentially, we need "core and aggregate" measurements. Obviously, the chemical composition/structure issue arises in these cases as well. This issue of properties and agglomeration affecting dosage rates for *in vitro* studies was covered in a recent review paper: J.C Teeguarden, et al, *Toxicological Sciences*, (2007) **95** 300.

Dr. Canady:

I don't know.

Dr. Cassell:

Surface charge, surface area, surface chemistry, geometry, and solubility are properties that come to mind for aggregates and agglomerates.

Dr. Lahann:

Most properties are depending on the dispersion state of the nanoobjects. Some times, individual nanoobjects can self-assemble into well-defined structures leading to novel functions and properties.

Dr. Murashov:

Transport properties will depend greatly on the state of aggregation/ agglomeration. Specific examples are
  a) diffusion coefficient;
  b) precipitation rate;
  c) surface deposition rate (lungs, skin);
  d) rate of penetration of filters, membranes, fabrics;
  e) translocation rates *in vivo*.
Effect of aggregation/agglomeration on **biological activity** will be determined by the mechanism of biological activity. For example, for poorly soluble low toxicity nanomaterials if aggregation/agglomeration results in changes in the total surface area available for interaction with biological species, then the biological activity of such materials is expected to change. For example, pulmonary exposure of rats to a well dispersed a sample of ultrafine carbon black was more toxic and inflammatory than an equal dose of a less dispersed sample (Shvedova et al., 2007).

Dr. Murday:

  a) Buoyancy (size/weight); capillarity; Ostwald ripening; grain growth; pore size distribution; hybrid structures – link a variety of different NP each with different properties (nano Au color in solution as an example); electrical/thermal transport in compact; mechanical behavior of compact; supported catalysis.

Dr. Small:

Agglomeration may affect size distributions from indirect methods such as DLS and surface area measurements. In addition agglomeration will also affect properties such as respirability, movement in environment and possible uptake.

Dr. Warheit:

No specific comments provided.

Question 7: *Are there routine manipulations of nanoscale materials that result in physical-chemical properties changes or other defining characteristics (e.g., surface modifications of nanotubes to enhance solvent dispersibility)?*

Dr. BelBruno:

Obviously, the cited case of surface modification is the main issue here. As may be inferred from responses to previous queries, such modification necessitates re-evaluation of the nanoparticle properties. Surface modifications include functional groups directed towards other applications, for example, to stealthly transport materials in biological systems. Usenko, et al, in an attempt to establish a rapid screening assay, have shown that $C_{60}(OH)_{24}$ was "significantly" less toxic than C_{60}. (*Carbon*, (2007) **45** 1891)

Dr. Canady:

I don't have specific knowledge of this but there are surely lots of data being developed and discarded in development of materials now that would speak to this. There is probably not a finite set or a small list of key manipulations, and the manipulations that result in relevant change will vary based on the material and the matrix or setting it is used in.

Dr. Cassell:

Yes, there are a number of physical and chemical treatments that can alter the defining characteristics of nanoscale materials. Vacuum treatment, thermal annealing, drying can all drastically influence the defining characteristics of nanoscale materials.

Dr. Lahann:

Fundamentally, dispersion requires the offset of interparticulate interactions with solvent/particle interactions. Changing the surface chemistry is an effective way of changing the surface energy of the particle and can therefore be used to tune the balance between these competing interactions. Similarly, change the solvent system can have similar effects. Finally, the application of electrical or magnetic fields may be used to tune dispersibility.

Dr. Murashov:

My understanding is that depending on application some nanomaterials are routinely modified to
   a) improve biocompatibility (e. g. surface functionalization of medical nanoapplications with polyethylene glycole);
   b) protect from dissolution and improve photoluminescence by surface passivation (e. g. coating of quantum dots with ZnS in CdSe/ZnS and/or organic ligands);

c) improve dispersibility and reduce agglomeration (e.g. use of surfactants to disperse carbon nanotubes).

Dr. Murday:

b) "Routine" varies depending on NS and application, i.e., it is not clear that the word "routine" is valid in this context.
c) It is likely that most NS will require a "shell" to make them compatible with their intended application(s). The creation of that shell can happen by many variants of either gas (including low pressure – vacuum) or liquid deposition. Since the environment interacts directly with the outermost shell, it should receive careful attention.

Dr. Small:

Materials may be modified in the purification process i.e. CNT can be acid washed to remove catalyst which may functionalize surface. Chemicals may be added during production to modify surface characteristic to prevent aggregation. Milling will change the surface characteristics.

Dr. Warheit:

No specific comments provided.

Question 8: *How should physical-chemical property data be prioritized for the NMSP? Based on availability, effect (toxicity or exposure criteria), or other factors?*

Dr. BelBruno:

The preferred method should be toxicity. However, it is not at all clear that such data would be available for each nanoparticle. The best approach would be to begin with those nanomaterials for which we do have data and construct what could be a prototype nanomaterial MSDS. Obviously, the test cases would be "pure" materials such as carbon nanotubes or TiO_2 or many of the quantum dot materials. However, we must establish consistent (and meaningful) standards for *in vitro* testing.

Dr. Canady:

Not sure what is meant by priority in this case but there is probably a set that is relevant to decisions and a broader set where the intention is to build knowledge of what might be relevant to decisions. Variables that would be informed by the data might include

a) Classification for regulatory purposes like things that would change inventory status or inclusion in a SNUR,
b) Fate and transport (and exposure or bioaccumulation or magnification or sequestration in odd places as a result), and
c) Toxicity or environmental effect

The degree to which data inform decisions in consideration of these variables would seem to be the main prioritization criteria.

Dr. Cassell:

The NMSP should take a careful and comprehensive approach to understanding nanoscale materials. Priortization of data should be made based upon the detailed context of the question and the specific situation. There are also many poor sources of information or information that may not be valid.

Dr. Lahann:

Based on effect.

Dr. Murashov:

Nanoscale Materials Stewardship Program goal is to better understand the potential risks and benefits of nanotechnology, therefore, physical-chemical property data should be prioritized according to its relevance to risk assessment and risk management of nanomaterials. However, it is also important to collect all the available information relevant to risk assessment (hazard and exposure) and risk management.

Dr. Murday:

a) Risk is roughly the product of exposure and harm.
b) Free standing nanostructures can lead to exposure; embedded might be released if not covalently bonded, otherwise parts of matrix will likely be attached; "thin film" nanostructures likely will never be available for incorporation into living systems.
c) Higher priority properties might be:
  a. Chemical reactivity
  b. Solubility/Partition (largely governed by surface properties and Hamaker constants)

Dr. Small:

The prioritization of the physical-chemical property data to be relevant needs to be based first on measurements that can be made in a reproducible manner both intra - and inter - laboratory. Next once these methods have been identified then their effect/application to risk assessment, (toxicity, exposure etc.) needs to be applied to the prioritization process.

Dr. Warheit:

With regard to human health effects or ecotoxicity testing, there are however, certain generalized points relating to material characterization for all nanoparticle-types that should be emphasized. These are the following:

Many scientific organizations or task forces have strongly recommended that toxicologists adequately characterize physicochemical properties of the nanoparticle-types that are being evaluated for hazard testing. However, too often this recommendation becomes a "laundry list" of physicochemical characteristics and does not have adequate prioritization. As a consequence, in order to adequately describe the physical characteristics of the nanoparticle-type being evaluated, I would recommend that toxicologists characterize the following (prioritized) physicochemical properties prior to conducting hazard studies with nanoparticle-types:

a) Particle size and size distribution (wet state) and surface area (dry state) in the relevant media being utilized – depending upon the route of exposure;
b) Crystal structure/crystallinity;
c) Aggregation status in the relevant media;
d) Composition/surface coatings;
e) Surface reactivity;
f) Method of nanomaterial synthesis and/or preparation including post-synthetic modifications (e.g., neutralization of ultrafine TiO2 particle-types);
g) Purity of sample;

This represents a focused approach concomitant with a minimum, standardized assessment of physicochemical properties that should be investigated prior to the development of toxicity testing with nanoparticles.

Additional points to consider for material characterization:

a) Depending upon the exposure scenario, it may be appropriate to conduct assessments of nanoparticle characteristics in both the wet state (in the relevant media) and the dry state. Therefore a BET surface area measurement may not suffice – but should be carried out in parallel with different types of measurements such as cryo TEM (dry state) and DLS (dynamic light scattering - particle sizing in the wet phase).
b) The chemical composition of the nanoparticle core vs. shell (hard or soft) should be evaluated.
c) In this regard, the surface chemistry could be specific for each nanoparticle-type.

Below, I have listed 5 recently-published nanoparticle toxicity studies from our Laboratory concomitant with the specific physicochemical characteristics that were identified from each of the studies. Accordingly, we have concluded that characteristics of nanoparticle surface reactivity appeared to be the best predictors of in vivo pulmonary toxicity in rats. However, it should be pointed out that surface reactivity evaluations are particle-type specific (e.g. Vitamin C assay for TiO2 particles; erythrocyte hemolysis for silica particles). Therefore, the same tests for surface reactivity cannot be utilized for each nanoparticle-type.

A Listing of Material Characterization Methods utilized in the 5 studies listed below

1) "Pulmonary Instillation Studies with Nanoscale TiO2 Rods and Dots in Rats: Toxicity is not dependent upon Particle Size and Surface Area". Warheit DB, Webb TR, Sayes CM, Colvin VL, and Reed KL. . Toxicol Sci. 91: 227-236, 2006

Material characterization employed in this study:
   a) synthesis method
   b) crystal structure
   c) particle size
   d) surface area
   e) composition/surface coating
   f) aggregation status
   g) cryo TEM
   h) crystallinity
   i) purity (TGA)

2) "Pulmonary bioassay studies with nanoscale and fine quartz particles in rats: Toxicity is not dependent upon particle size but on surface characteristics". Warheit DB, Webb TR, Colvin VL, Reed KL and Sayes CM. Toxicol Sci. 95:270-280, 2007;

Material characterization employed in this study:
   a) synthesis method
   b) crystal structure/crystallinity (XRD)
   c) median particle size - particle size (range)
   d) purity (% Fe content)– ICP-AES
   e) surface area
   f) TEM
   g) aggregation status
   h) purity
   i) surface reactivity (erythrocyte hemolysis)
   j) reactive oxygen species (ESR)

3) "Pulmonary Toxicity Study in Rats with Three Forms of ultrafine-TiO2 Particles: Differential Responses related to Surface Properties". Warheit DB, Webb TR, Reed KL, Frerichs S, and Sayes CM. Toxicology 230:90-104, 2007;

Material characterization employed in this study:
   a) crystal phase
   b) median particle size and size distribution in water and PBS
   c) pH in water and PBS
   d) surface area (BET)
   e) TEM
   f) aggregation status
   g) chemical (surface) reactivity – (Vitamin C assay)
   h) surface coatings/composition, purity

4) Assessing toxicity of fine and nanoparticles: Comparing in vitro measurements to in vivo pulmonary toxicity profiles Sayes CM, Reed KL, and Warheit DB. Toxicol Sci, 97:163-180, 2007.

Particle-types utilized in this study:
   a) Fine-sized carbonyl iron
   b) Fine-sized crystalline silica
   c) Fine-sized amorphous silica
   d) Nano ZnO
   e) Fine ZnO

Particle characterizations conducted both in the "dry state" and "wet state"

Material characterization employed in this study:

Particle characterization in the dry state
   a) particle size
   b) surface area
   c) density
   d) calculated size in dry state (based on surface area determinations)
   e) crystallinity
   f) purity

Particle characterization in the wet state
   a) particle size in solutions – PBS, culture media, water
   b) average aggregated size in solutions
   c) % distribution
   d) surface charge
   e) aggregation status

Conversion and comparisons of in vitro and in vivo doses for dosimetric comparisons

   5) Comparative Pulmonary Toxicity Assessments of C60 Water Suspensions in Rats: Few
      Differences in Fullerene Toxicity In Vivo in Contrast to In Vitro Profiles.   Sayes CM,
      Marchione AA, Reed KL, and Warheit DB. Nano Lett. 2007 Aug;7(8):2399-2406.

Material characterization employed in this study:
   a) particle size and size distribution
   b) surface charge
   c) crystallinity
   d) TEM
   e) Composition
   f) oxidative radical activity (ESR measurements)
   g) surface reactivity (erythrocyte hemolytic potential)

Nanoscale manufacturing and processing

       The number of manufacturing and processing methods for generating nanoscale material
continues to grow and become more sophisticated. The two primary areas for this discussion

include physical reduction methods (milling) and engineering methods (e.g., particle stabilization, vapor deposition, self assembly).

Question 9: *What are the common processes used to manufacture nanoscale materials?*

Dr. BelBruno:

The list in the paragraph above covers the general "bottom up" and "top down' methods.

Dr. Canady:

A list was developed in the ICON workshop last year at NIH – I don't have the full list, but it is extensive.

Dr. Cassell:

Solution based methods are quite common and include a number of approaches (self-assembly, colloidal preparation), chemical vapor deposition is also used extensively. All techniques conventionally used in organic and inorganic synthesis are also adapted for nanoscale materials manufacture.

Dr. Lahann:

Organic materials: self-assembly
Inorganic materials: CVD
Polymers: staged synthesis, self-assembly, suspension polymerization, grafting, electrified jetting
Composites: similar to polymers

Dr. Murashov:

The draft ISO TC229 Technical Report on "Health and Safety Practices in Occupational Settings Relevant to Nanotechnologies" lists the following categories of major processes used to manufacture nanoscale materials:
   a) Gas phase processes such as flame pyrolysis, high temperature evaporation and plasma synthesis;
   b) Vapor deposition;
   c) Liquid phase methods: colloidal, self-assembly, sol-gel;
   d) Supercritical fluid;
   e) Electro-spinning for polymer nanofiber synthesis;
   f) Mechanical processes including grinding, milling and alloying;
   g) Bioreactors.

Dr. Murday:

   a) Since NS, by definition, have large surface to volume ratios, quality manufacturing processes must utilize a controlled environment.

b) For large scale bulk NS the processes are largely either a variant on colloid chemistry or gas phase nucleation and growth. Fumed silica and carbon black are examples of the latter – both can be NS and have been made in volume for decades.

Dr. Small:

Common methods would include: lithography, vacuum and spray coating, CVD, Vapor deposition, Plasma vaporization, laser ablation, arc-discharge, gas-phase catalytic growth of CNT from carbon monoxide, and Sol gel. There are of course many "variations" on these methods some of which are proprietary. In the case of the more complex materials these would probably start with one of the above and then have proprietary additional steps.

Dr. Warheit:

No specific comments provided.

Question 10: *How are processes used to produce specific characteristics or properties?*

Dr. BelBruno:

The size of synthesized particles can be controlled through the chemistry, either by limiting reagents or by the specific conditions of the chemistry (heat, solvent, reaction mechanism, etc). To the extent that properties reflect size (and surface area), then the properties are controlled by the chemistry. Of course, that relationship (size-properties) is not yet well-defined. Similarly, self assembly can be chemically controlled and the surface characteristics, therefore, influenced by the process. Finally, chemistry can be used to modify or functionalize the particles. We know that functionalized nanotubes, for example, have different properties from the naked nanotubes.

Dr. Canady:

I don't know.

Dr. Cassell:

Self assembly for instance can be used to control nanoparticle diameter. Polymerization techniques (stoichiometric control, temperature, pressure, etc.) can be used to control engineered polymer systems. Thermal/chemical treatment can be used to increase surface area or surface chemistry. There are a wide variety of techniques borrowed from conventional disciplines that are used in the manipulation of nanoscale materials.

Dr. Lahann:

By exactly controlling chemical composition and structure of nanoparticles/nanoobjects, a specific characteristic or property can be achieved. For instance, in the case of CdSe nanocrystals, the particle diameter defines important optical properties and developing processes, which can generate monodisperse particles with defined diameters, will give a unique function.

Dr. Murashov:

This depends on the process, materials and application.

Dr. Murday:

a) After the formation of most NS it will be necessary to tailor the surface to make the NS compatible with further processing. The tailored surfaces can be thought of as a shell. There may be several shells as we progress toward "smart" NS.
b) As an example, the first shell of a semiconductor QD is designed to trap the electron-hole pair in the core, a second shell might have a therapeutic function and a third shell a recognition function.
c) Processes used to produce the shells will likely vary considerably, but would again be mostly variants on colloid/gas phases processing.

Dr. Small:

No specific comments provided.

Dr. Warheit:

No specific comments provided.

Question 11: *Which methods reduce particle size but do not result in property changes? Which methods reduce particle size and result in property changes?*

Dr. BelBruno:

See response to question 10.

Dr. Canady:

First, the question should be about change not reduction. Size going up from 50 to 200nm will cause different disposition effects in the body, so increase in size in the nanoscale range is relevant as well.

But I don't understand this question. Size is a property that has relevance in biology on its own. If "property" as a function of size means lack of biological or environmental impact, then how

could you know that properties are not changed without testing? How could we know the answer to this question unless the answer is that all methods to change size will result in some unknown likelihood of a change in biological or environmental response? This is a smaller set of the question of whether you can assume that any modification will have no effect (by limiting the modification to "size change" rather than "any modification").

Dr. Cassell:

This depends on the system under question. Cutting a carbon nanotube in half reduces its particle size, but does not greatly effect other properties. Reducing gold particle diameters dramatically alter melting point for instance.

Dr. Lahann:

If a material's change in particle size results in fundamental property changes, the outcome is more a function of the material itself and less of the method used to reduce the size. However, the process used to reduce the size of a material may determine polydispersity and may still be influencing properties.

Dr. Murashov:

If the reduction in size results in changes in properties of interest, then it does not matter how the reduction in size was achieved. If properties of interest also depend on surface characteristics, then the production method can affect these properties and additional steps such as surface passivation might be required (see also answer to question 7).

Dr. Murday:

   a) The question is not meaningful – all methods may/may not change properties.
   b) Processes without a controlled environment are likely to lead to wider variation in surface composition and thereby different behaviors. For instance, the technique of ball milling larger particles into smaller frequently is inadequately controlled; compacted powder studies have suffered from inability to reproduce results.
   c) To control size, processes growing NS will need to have a nucleation event separate from the growth step. If nucleation and growth proceed in parallel, there is an inevitable distribution in NS size.

Dr. Small:

Many of the production methods are bottom up so this question does not apply to those. In general the property change results for the nanoscale characteristics of the materials not on how they are produced. Not sure what this question refers to.

Dr. Warheit:

No specific comments provided.

Impurities

Impurity content is a growing area of interest in nanotechnology due to improved performance observed in some cases (e.g., solar cells and semiconductors) and deleterious effects observed in others (e.g., quantum dot quantum computers). The confounding effects that impurities have with respect to toxicological endpoints are also being studied (National Nanotechnology Initiative 2006 Environmental Health and Safety research report[9]).

Question 12: *How important are impurity identity and impurity levels to the understanding and characterization of nanoscale materials?*

Dr. BelBruno:

Impurity level and identity are important factors, since the impurities are also nanometer scale and their own properties add to the difficulty in assessing the impact of the target particles. Again using nanotubes as an example, the catalytic metals that are sometimes incorporated in the final product need to be identified, quantified and, often, removed. This has been accomplished in a number of different ways. One example is provided by Y. Wang, et al, *Chemical Physics Letters* (2006), **432**, 205.

Dr. Canady:

Impurity identity is critical and not clear at this point. It could be defined relative to the set of nanoscale properties of interest which would also be the specification of the material, or of the nanoscale particle. Something could be an impurity if it has slight topographic variation from the optimal and conversely, something may not be an impurity if it has dramatically differing composition but similar enough structure so that the effect does not depend on composition.

Impurity could also be defined based on unwanted side effects of a variation in nanoscale material even if the nanoscale material that is "impure" (in the sense of its unwanted side effects) is "pure" with respect to its intended effects.

Impurity could also just be a solvent or carryover from the manufacturing in the typical sense, such as the PAHs in CNTs.

The need to measure impurity level is a function of effect and would be case-dependent.

Dr. Cassell:

Extremely important. I would say that impurities become more relevant as you scale your system to smaller dimensions. Impurities could be even more important in the context of toxicological effects.

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[9] http://www.nano.gov/NNI_EHS_research_needs.pdf

Dr. Lahann:

Depending on the application, the effects can either be hugely important or can be neglected. However, impurities always introduce an additional source for structural variability into a nanomaterial.

Dr. Murashov:

There is a need to clarify what is meant by "impurity". Term "impurity" when it is applied to nanomaterials is more similar to molecular chemicals (e. g. organic polymers) than to macroscopic materials with a high degree of ionic or metallic bonding (e.g. SiO_2, Si, NaCl). Specifically, target nanomaterials can be contaminated by other nanomaterials and chemicals (example is a raw carbon nanotube material composed of a mixture of carbon nanotubes and nanoscale metal catalyst particles). Nanomaterials can be also "contaminated" by atomic/molecular species covalently bonded to nanomaterials. If such a "defect" or "contamination" results in significant changes of nanomaterial properties, it could be considered a new nanomaterial rather than "contaminated" or "defective", just as replacement of an atom in a molecule for a different chemical element creates a new molecule/chemical. Presently, in many instances nanomaterials are produced as mixtures of nanoparticles with a range of particle sizes, shapes and chemistries, which is, to some degree, similar to a mixture of oligomers obtained during polymer synthesis.

The importance of "impurity" or "impurity" level (or the amount of other molecules and nanomaterials in the mixture) for a particular application will be strongly dependent on that application. The importance of "impurity" or "impurity" level on risk of a target nanomaterial will be strongly dependent on risk-related properties of the "impurity" and the target nanomaterial.

Dr. Murday:

a) Impurity identify and level can be very important, i.e., doping of a semiconductor NS. The NS size is sufficiently small that a single dopant atom will have dramatic impact on its electrical properties. Doping at the nanoscale might be better accomplished by injecting charge through surface bond compensation.
b) CNT manufacture provides an excellent case study in quality control (or lack thereof). Figure A shows the results of an analysis of eight different nominal CNT samples – the worst has only 40% carbon (as a fraction of C, H, O; all other elements were excluded from the analysis), and that carbon might not be NT.

Dr. Small:

This is a critical area when characterizing these materials. For example, the catalyst in NT production is nanosized metal (Fe, Ni) particles. Some studies indicate that these may be more toxic than the NTs. The ability to determine the amount and the physical-chemical character of the impurities contained in ENM relates directly to understanding and evaluating exposure and risk.

Dr. Warheit:

No specific comments provided.

Question 13: *Are there routine purification procedures that can effectively control or remove impurities, when desirable, for certain classes of nanoscale materials?*

Dr. BelBruno:

For nanotubes, the response is yes (see Q12). Removing impurities from inorganic materials is more difficult because procedures could also affect the desired particles.

Dr. Canady:

I don't know.

Dr. Cassell:

Yes. There are many techniques that are borrowed from those known in a number of disciplines (chemistry, materials science, biology) and used in nanoscale materials manipulation. They are too numerous to list here, but include techniques such as annealing, chemical treatment, separation, chromatography, sedimentation, plasma treatment.

Dr. Lahann:

Processes to remove impurities from nanoscale materials are often available.

Dr. Murashov:

The following broad classes of purification processes can be distinguished:
   a) chemical modification to reduce heterogeneity;
   b) dissolution of ionic, molecular and particulate species from target nanomaterials (e.g. acid-washing has been reported as a means of removing metal catalytic particles from raw carbon nanotube material);
   c) mobility sizing using difference in mobility of nanomaterials of different sizes, masses, charges and shapes in gas and liquid phases, across membranes and subject to force-fields.

Dr. Murday:

   a) Chemistry provides a rich source of purification processes that can be exploited for NS. One limitation could be difficulty in sustaining solubility/suspension in fluid media.

Dr. Small:

Acid washing for CNT along with other steps has been used for purification of CNTs. There are several lab-based methods these are not routine.

Dr. Warheit:

No specific comments provided.

Obtaining characterization data

Determining identity, quantifying the nanoscale particle range, and measuring physical-chemical properties for that identity and particle range are essential to the characterization of nanoscale materials. Because of the challenges associated with size, shape, surface characteristics, and possibly other aspects of nanoscale materials, an evaluation of existing measurement techniques is critical to nanoscale material characterization. The National Nanotechnology Initiative report stated that "...Accurate and useful measurement techniques are also important because agglomerated nano materials may either retain or lose their emergent properties - or take on new properties - thus affecting the potential biological response."

Question 14: *Are validated methods available for the different categories of nanoscale materials?*

Dr. BelBruno:

Particles are easily sized using commercially available equipment. This measurement is a bit more difficult (larger uncertainty) for coated nanoparticles. Force and electron microscopy may also be used to measure the size of particles. These are essentially universal, but tedious, methods. X-ray spectroscopic techniques can be used to identify metallic impurities (in many electron microscopes). Surface area measurement is also a well-developed technique. (see Q4)

Dr. Canady:

No specific comments provided.

Dr. Cassell:

I am unaware of validated methods for nanoscale materials in particular, but there may be methods (e.g. ISO) that are inclusive of nanoscale materials.

Dr. Lahann:

Some validated methods are available, but further efforts are needed.

Dr. Murashov:

There are no validated methods specific to engineered nanoparticles (NIOSH 2006). There are validated methods for materials that exist in the nanometer scale, such as welding fume, carbon black; but these methods do not discriminate by size. There are some methods that have been validated for the larger (bulk) form of nanomaterials that may be applied to the nano form; for instance dust sampling with a cyclone (ISO, 2007); sampling for a specific element with analysis by NIOSH 7300 (NIOSH, 1994); fiber sampling and analysis by Transmission Electron Microscopy (TEM). These methods have not been validated for nanoscale material, but there is potential for reapplication and they can provide useful information for evaluating occupational exposures with respect to particle size, mass, surface area, number concentration, composition, and surface (NIOSH, 2006). The challenge is demonstrating that the data generated by the measurement (the metric) was created by the engineered nanoparticle rather than by incidental nanoparticles of similar chemical composition. Variations of the sampling process for mixtures of nanoscale and fine TiO_2 are being tested (NIOSH, 2005). One of the challenges of that method is collecting enough material for the first mass-based step in the analysis. Often the electron microscopy analysis is used to verify that the material collected has the expected elemental composition and is in the nanometer size range though there is always some degree of agglomeration.

Dr. Murday:

   a) MS and light scattering are the closest to validated.
   b) NIST and NCL are working this issue – EPA should partner.

Dr. Small:

There are several methods under development be few in publication. One I am aware of is IEEE P1650 standard test method of meas. of electrical properties of CNTs adopted in 2005.

Dr. Warheit:

No specific comments provided.

Question 15: *Are there techniques that can be universally applied?*

Dr. BelBruno:

See response to question 14.

Dr. Canady:

No specific comments provided.

Dr. Cassell:

I would think so, yes. Especially the more generic particle characterization techniques (surface area, particle size determination, etc.) that have been utilized in other materials characterization work.

Dr. Lahann:

Particle size and shapes can be characterized using Transmission electron microscopy, scanning electron microscopy and scanning probe microscopy independent of the materials composition.

Dr. Murashov:

The collection and analysis of an air sample by TEM is an example of a universal technique which can be applied across several types of nanomaterials.

Dr. Murday:

a) In a broad context, there are no techniques that provide all the necessary information, all the time. NS can be found in free form and embedded in matrices. The NS itself is likely to have composition/structure that varies with distance from its center.
b) Light scattering is likely to be useful for size/shape measurement. It might be useful/important to extend the photon energy in the far UV to make the wavelength more comparable to the NS size (however, this may invoke a problem for the optics).
c) Mass Spectrometry has been developed to handle very large molecules – 100s of KDa. Fragmentation patterns can illuminate substructures. This technique could prove very useful in probing core/shell NS.
d) MS and light scattering require multiple copies of a NS and yield ensemble averaged results. Techniques to probe individual NS properties are also very useful. Some variant on HRSEM with energy dispersive analysis for composition, coupled with AFM, is likely to be the most versatile.
e) SERS might be developed with enough sensitivity to analyze one or a few NS for a vibrational approach to composition.

Dr. Small:

Not that I am aware of. Perhaps some of the microscopy methods could be used to characterize multiple classes.

Dr. Warheit:

No specific comments provided.

Question 16: *For small quantities of materials, are there sampling, handling, and collection techniques as well as sample integrity, accuracy and precision QA/QC methodologies available?*

Dr. BelBruno:

Many nanoparticles are available from commercial suppliers (quantum dots, fullerenes, nanotubes, inorganic cluster molecules), with specified purity levels and chemical composition. Clearly, commercial suppliers can arrange QA procedures and we should look to these methods as a means of introducing standards.

For example, from American Elements (http://www.americanelements.com/fenp.html): "Iron (Fe) Nanoparticles, nanodots or nanopowder are spherical or faceted high surface area metal nanostructure particles. Nanoscale Iron Particles are typically 20-40 nanometers (nm) with specific surface area (SSA) in the 30 - 50 m^2/g range and also available in with an average particle size of 100 nm range with a specific surface area of approximately 7 m^2/g. Nano Iron Particles are also available in Ultra high purity and high purity and coated and dispersed forms."

Dr. Canady:

No specific comments provided.

Dr. Cassell:

I would say that there may be generic techniques published in the technical literature, but these are probably not standardized by a leading organization. Quality control procedures for nanoscale materials are largely dependent upon the organization that handles the materials and most of these are based upon the needs of that particular organization.

Dr. Lahann:

Some specialized procedures are available, but further efforts are needed.

Dr. Murashov:

At this point, the greatest QA/QC challenge is the lack of methods specific for nanoscale particles and the lack of reference materials that accurately represent the material being evaluated.

Dr. Murday:

   a) The traditional handling of chemicals provides proven precedents. But they will have to be adapted to the broader range of size/properties incumbent in NS.
   b) Testing on NS shows (at least in some circumstances) that they can be collected effectively on filters – van der Wall forces or surface charges attract to the filter material; NS are small enough that fluid drag forces are small and don't displace them from the filter.

Dr. Small:

No. I think best-practices methods (handling of ultrafines) are used although there are several efforts by government agencies and industry to develop safe handling practices for ENMs that would apply to "small" quantities.

Dr. Warheit:

No specific comments provided.

Question 17: *What is the status of standardization efforts? Are these efforts focused on broadly applicable characterization methods or category-specific methods?*

Dr. BelBruno:

Mostly category specific, as the response to Q19 indicates.

Dr. Canady:

No specific comments provided.

Dr. Cassell:

NIST is active in generating standards for single walled carbon nanotubes see: http://polymers.nist.gov/Nanotube3/Agenda.pdf. There are a variety of techniques described for characterizing single walled carbon nanotubes.

Dr. Lahann:

Depending on the field and material, standardization efforts vary and further activities are needed.

Dr. Murashov:

Standardization efforts are focused on both broadly applicable characterization methods and category-specific methods. For example, the ISO TC 229 (Nanotechnologies), Working Group 3 (Health, Safety and the Environment) is working on five projects:
   a) Technical Report "Health and Safety Practices in Occupational Settings Relevant to Nanotechnologies" – broadly applicable characterization methods;
   b) International Standard "Endotoxin test on nanomaterial samples for in vitro systems" – broadly applicable;
   c) International Standard "Generation of silver nanoparticles for inhalation toxicity testing" – specific to silver nanoparticles;
   d) International Standard "Monitoring silver nanoparticles in inhalation exposure chambers for inhalation toxicity testing" - specific to silver nanoparticles;

e) Technical Report "Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment" - broadly applicable.

The ISO TC 229 Working Group 2 (Metrology and Instrumentation) is working on a number of standards for characterizing carbon nanotubes.

Dr. Murday:

No specific comments provided.

Dr. Small:

Physical standards: Standardization efforts are in the process of ramping up both from an activity as well as a funding view point. Government, industry, and academia, in the US as well as other areas like Europe are in the process of defining and measuring candidate materials. Efforts are focused on both category-specific i.e. CNT and on broader definitions such as metal oxides or on schemes used for classifications of materials like toxicity, commercial impact, and environmental impact.

Documentary Standards (methods): As with the physical standards these efforts are also increasing although development of "standard methods, in most cases, requires the availability of well characterized physical standards.

Dr. Warheit:

No specific comments provided.

Question 18: *What alternative or innovative methods or technologies can be applied to nanomaterial analysis?*

Dr. BelBruno:

No specific comments provided.

Dr. Canady:

No specific comments provided.

Dr. Cassell:

Atomic force microscopy along with some of the latest scanning probe microscopy techniques could be useful for nanomaterials analysis. I'm unsure if standards for characterizing with these techniques have been set.

Dr. Lahann:

Transmission electron microscopy, scanning electron microscopy and scanning probe microscopy, fluorescence microscopy, X-ray microscopy.

Dr. Murashov:

Some examples of alternative or innovative methods or technologies that could be applied to nanomaterial analysis as part of safety and health assessments are:
a) A number of instruments providing on-line size-resolved chemical speciation of aerosols, such as aerosol mass-spectrometry, have been developed. These techniques can provide compositional information on single particles or ensembles of size-selected particles in real time.
b) Size distribution and number concentration of nanoparticles in liquid phase can be characterized using light scattering, laser diffraction, size exclusion chromatography, acoustic techniques and field flow fractionation (Powers et al, 2006), while space-resolved spectroscopic techniques can be useful in obtaining information about chemical composition and structure of nanoparticles.

Dr. Murday:

a) ASTM interlab standards not yet in place.
b) Tip characterization for proximal probes is a major challenge.
c) Absence of standard materials another factor.
d) Lab on a chip and variants on microarrays might offer new approaches that could be readily adapted to field use.
e) To the extent that NS impact on living systems is paramount, cell based microarrays (being developed for drug candidate screening) might provide a "canary on a chip."

Dr. Small:

There are many novel technologies, e.g. microscopy-based, as well as others, which are applicable to nanoscale regimes. Is the EPA looking for methods that are likely to be widely used (cheap and fast) or for very specific research areas? Many of these methods are currently difficult and expensive to make and are confined to laboratory studies some may eventually become routine others will not. Examples of some techniques include: super-resolution optical, 3D chemical tomography, TGA on chip, size-exclusion chromatography.

Dr. Warheit:

No specific comments provided.

Modeling

Empirical modeling can be a useful approach to predict physical-chemical properties when experimental data are not known or ascertainable. The initial problem with modeling is that, to accurately predict property endpoints for a given category of substances, there must be some experimental data available in the tool's database for at least some representative substances in that category. For newly discovered or studied materials, the minimum but necessary quantity and type of experimental data often is not available to sufficiently populate a tool's database and allow accurate prediction by the tool. Some estimation methods have been developed for specific property endpoints, but many others are lacking.

Question 19: *Are there models that are currently used to obtain property data for nanoscale materials? For which properties and which nanoscale materials?*

Dr. BelBruno:

I believe that we need to have a long-term view for this. Simulations provide agreement with already established data, but are not predictive. Quantum mechanical calculations provide a basis for the emission in quantum dots. However, structure function type relationships of the type used in the pharmaceutical industry do not exist.

Dr. Canady:

I don't know.

Dr. Cassell:

There are some atomistic approaches that have been used to predict properties of nanoscale materials and some of these models have been extended to molecular dynamics and mesoscale modeling approaches. I am not an expert in the field, but I am aware of these techniques. The properties modeled are typically physical in nature such as mechanical, thermal or electrical in nature. Primarily these approaches are utilized to understand transport phenomena.

Dr. Lahann:

A series of different approaches is currently under development depending on specific materials categories.

Dr. Murashov:

Only few risk-related models exist that are currently used to obtain property data for nanoscale materials. Examples are:
   a) Several models currently used to obtain property data for nanoscale materials are models based on aerodynamic properties of nanoparticles such as penetration through filter media, coagulation and deposition in the lung (Lee and Liu, 1982; Maynard and Kuempel, 2005; ICRP, 1994).

b) Research data indicate that health hazard properties of poorly soluble, low-toxicity nanoparticles can be modeled using their total surface area (Maynard and Kuempel, 2005; Duffin et al., 2007).

Dr. Murday:

a) It is possible to extrapolate from smaller (atomistic) and larger (continuum) scale models, but caution must be exercised.
b) First principle models of CNT were able to predict accurately many of their properties well before the experimental community could measure them (see Figure C). However, CNT is a special case of NS – CNT is all C, has only on type of bond, and has a high degree of symmetry.

Dr. Small:

No specific comments provided.

Dr. Warheit:

No specific comments provided.

Question 20: *Has any validation work been conducted that compares predicted values with measured data? For which properties and which nanoscale materials?*

Dr. BelBruno:

See response to question 19.

Dr. Canady:

NCL has published standard methods through ASTM for some assays that have validation data.

Dr. Cassell:

Individual carbon nanotube based thermal conductivity has been predicted by theory and subsequently validated using experimental approach. Prof. Arun Majumdar's group at UC Berkeley performed these elegant experiments.

Dr. Lahann:

Some of the models have been validated. A good example is the work of Nick Kotov and Sharon Glotzer at University of Michigan that experimentally validated the formation of nanoparticle sheets (Science 2006). More work is needed.

Dr. Murashov:

Validation work has been conducted for data related to penetration of filter media by nanoscale particles (see for example, Rengasamy et al., 2007).

Dr. Murday:

a) CNT model predictions have been verified by experiment (see Figure C).
b) QD model predictions have been verified by experiment.
c) Fluid flow in nanopores has been examined by Navier Stokes (continuum) and molecular dynamics approaches to understand the modeling limitations. Results have been partially validated by experiment.

Dr. Small:

No specific comments provided.

Dr. Warheit:

No specific comments provided.

Question 21: *Are there current significant characterization needs for which the NMSP should investigate model development?*

Dr. BelBruno:

See response to question 19.

Dr. Canady:

Yes – but an entire workshop or similar effort should be devoted to developing ideas and projects for modeling. The most immediate one may be developing a modeling approach that allows updating of the information needs relative to the decision needs. As more is learned about the materials and the properties that are most relevant to decisions there will need to be a process for adjusting the information requirements.

Dr. Cassell:

I'm not sure if there are aggregation/agglomeration models that could be used for various types of nanomaterials, but I see this as being a valuable method to augment characterization approaches. Any predictive tool that is useful in a contextual setting as opposed to idealized models would be extremely useful.

Dr. Lahann:

Specifically in the area of correlation between nanoparticle properties, such as size, shape, composition, and biological properties, such as cell uptake or cytotoxicity, there would be a great potential for model developments.

Dr. Murashov:

Examples of current significant characterization needs for which the NMSP should investigate model development are:
   a) Risk assessment models for nanoscale materials are needed. Specifically, models to extrapolate toxicity data from *in vitro* to *in vivo*; from *in vivo* to human; external exposure levels to internal dose should be further developed.
   b) There is a need to develop models correlating biomarkers of exposure with specific nanomaterials.
   c) There is also a need to determine if certain physico-chemical properties of nanoparticles are predictive of biological activity.

Dr. Murday:

   a) Chemical reactivity – partner with catalysis efforts.
   b) Toxicology – partner with NIH, NIOSH, NIST, NCL.
   c) Fate and effect in soils – a topic more closely aligned with EPA than other agencies.

Dr. Small:

No specific comments provided.

Dr. Warheit:

No specific comments provided.

Supporting Figures

Figures provided by Dr. Murday:

CNT	N	N Annealed	SL MWCNT	SL MWCNT Annealed	S MWCNT	S MWCNT Annealed	S DWCNT	S DWCNT Annealed	S SWCNT+ MWCNT	S SWCNT+ MWCNT Annealed
C (%)	38.1	32.7	60.8	39.3	69.1	58.7	66.0	43.2	44.7	34.3
H (%)	51.0	57.7	31.6	54.7	30.5	38.8	32.4	51.6	42.9	60.9
O (%)	10.9	9.6	7.6	6.0	0.4	2.5	1.6	5.2	12.4	4.8
Most Abundant Cluster	C_3OH_6	CH_3	C_5	$C^{2+} CH_3$	C_8H_n	C_8H_n	$C_{18}H_n$	$H CH_3$	COH_3	CH_3
Largest Cluster	C_8H_n	C_8H_n	C_8H_n	C_8H_n	C_8H_n	C_8H_n	$C_{18}H_n$	C_5H	$C_{18}H_n$	$C_{15}H_n$

Nishikawa   Kanazana Institute of Technology, Japan

Figure A

FIELD	PROPERTY	SCALE LENGTH		
ELECTRONIC	ELECTRON WAVELENGTH	10	--	100nm
	INELASTIC MEAN FREE PATH	1	--	100nm
	TUNNELING	1	--	10nm
MAGNETIC	DOMAIN WALL	10	--	100 nm
	EXCHANGE ENERGY	0.1	--	1nm
	SPIN-FLIP SCATTERING LENGTH	1	--	100nm
OPTIC	QUANTUM WELL	1	--	100nm
	EVANESCENT WAVE DECAY LENGTH	10	--	100nm
	METALLIC SKIN DEPTHS	10	--	100nm
SUPERCONDUCTIVITY	COOPER PAIR COHERENCE LENGTH	0.1	--	100nm
	MEISSNER PENETRATION DEPTH	1	--	100nm
MECHANICS	DISLOCATION INTERACTION	1	--	1000nm
	GRAIN BOUNDRIES	1	--	10nm
NUCLEATION/ GROWTH	DEFECT	0.1	--	10nm
	SURFACE CORRUGATION	1	--	10nm
CATALYSIS	LOCALIZED BONDING ORBITALS	0.01	--	0.1nm
	SURFACE TOPOLOGY	1	--	10nm
SUPRAMOLECULES	PRIMARY STRUCTURE	0.1	--	1nm
	SECONDARY STRUCTURE	1	--	10nm
	TERTIARY STRUCTURE	10	--	1000nm
IMMUNOLOGY	MOLECULAR RECOGNITION	1	--	10nm

Compilation of Panelists' Preliminary Comments

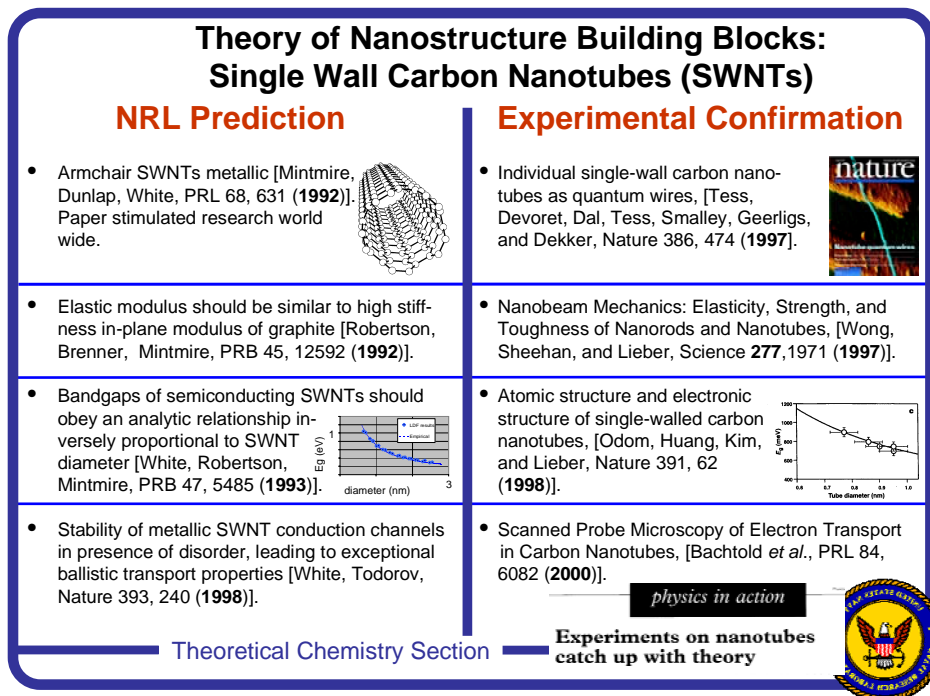**Figure B: Characteristic Lengths in Solid State Science Models**

Figure C

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"In summary, it was concluded that nanoparticle composition, dissolution, surface area and characteristics, size, size distribution, and shape are parameters needed for any target organ toxicity assessment. Depending on the type of toxicological study undertaken, other physicochemical parameters would also be required