Report on the
2016 Global Summit on Regulatory Science (GSRS16)
Nanotechnology Standards and Applications

7-9 September 2016

**U.S. National Institutes of Health Campus, Natcher Auditorium,
Bethesda, Maryland, USA**

1.0 Introduction

The global increase in applications of nanotechnology has produced medical and consumer products with new or enhanced functionality. Nanotechnology-enabled devices are faster, smaller, lighter, and smarter. In the medical field, there are new developments in precision and targeted medicines for improved therapeutic outcomes, while minimizing toxicity and patient side-effects. New *in vitro* diagnostics and *in vivo* imaging agents are under development for early disease detection, and there is continued improvement of advanced medical devices. These exciting advances in science, technology, and medicine provide an opportunity for additional regulatory science research to ascertain how different nanomaterial characteristics influence biological attributes, biocompatibility, biodistribution, safety, and efficacy. The 2016 Global Summit on Regulatory Science (GSRS16) was a collaborative effort among global regulatory, standards, and research agencies, and stakeholders from industry and academia. GSRS16 was aimed at highlighting recent advances in nanotechnology, educating the community on current research and regulatory perspectives, and developing consensus on the needs for nanotechnology standards. A major goal of this summit was to establish an inventory of research and standards critical to supporting the responsible development and safety evaluation of nanotechnology-based products. In this report, the general term "standard" encompasses consensus-based documentary standards, reference materials (RMs), and guidance documents.

2.0 Goals of GSRS16

The goals of the GSRS16 established by the Organizing Committee are as follows:

(1) Educate a broad group of stakeholders on the state of the art in nanotechnology science, measurement methods, and standards for regulatory applications.

Knowledge concerning regulatory applications has largely been limited to regulatory and standards agencies. The plenary sessions were designed to educate a broader group of stakeholders on the current state of science and relevant standards for regulatory applications, and on the current state of all nanotechnology standards worldwide.

(2) Identify the most immediate needs in nanotechnology science, measurement methods, and standards relevant to regulatory applications.

There has been significant progress over the last 15 years in advancing nanomaterial measurement methods, understanding the effects and utility of nanomaterials in biological systems, and developing RMs, documentary standards, and testing guidance documents for nanotechnology. This summit provided an opportunity to build on this existing knowledge in order to prioritize needs for science, measurement methods, and standards relevant to regulatory applications, and to enable much needed technologies in the pre-clinical, clinical, and food testing realms for societal benefit.

(3) Facilitate greater coordination between stakeholders in the development of standards.

It is recognized that the availability of standards for nanomaterial measurements intended for medical or food products under regulatory consideration is severely limited. Therefore, a principal objective of this summit was to gather stakeholders from government regulatory, research, and standards agencies, academic institutions, and industry to initiate a dialogue on enhancing coordination in the prioritization and development of standards for regulatory purposes.

3.0 Desired Outcomes of GSRS16

The two desired outcomes established by the Organizing Committee are as follows:

(1) Publication of a GSRS16 meeting report

The publicly available report includes a summary of the information generated by the panel sessions that captures and prioritizes needs for additional documentary standards, guidance documents, and RMs specifically targeted for regulatory applications of nanotechnology products. The report also addresses existing gaps in nanotechnology regulatory science across a broad spectrum of applications.

(2) Representative consensus for a centralized website

There are several websites that contain information on standards, e.g, the Nanotechnology Standards Database hosted by the American National Standards

Institute[1]. A new, centralized website containing links to all international standards is needed to consolidate this information at a single location. This website may be part of the European Union's Nanomaterials Observatory to be established and hosted by the European Chemicals Agency (ECHA).

Other potential outcomes of GSRS16 include the following:

(1) Publication of a journal article based on the findings of GSRS16
(2) Collaborative work among the community stakeholders in the development of key standards identified by GSRS16 participants
(3) A follow-up meeting on nanotechnology standards to maintain the momentum generated by the pre-GSRS15 and GSRS16 meetings

4.0 GSRS16 Program Overview

The first day of GSRS16 began with a plenary address by Robert Califf, M.D., Commissioner of the US Food and Drug Administration (FDA) and was followed by two plenary sessions, one with presentations by representatives of international regulatory agencies and the other with presentations on standards as defined in section 1.0 of this report. Day one concluded with a poster session. Days two and three of GSRS16 had two parallel sessions on different applications involving nanomaterials, including drugs, medical devices, food and food contact materials, and personal care products. At the end of each parallel session, a moderated discussion with a panel of the speakers was held to describe the needs for research and new standards to support regulatory decisions. Subsequently, there were brainstorming discussions with audience participation. Day three concluded with a plenary session summarizing the discussions in each of the parallel sessions. Morning and afternoon breaks, lunches, and a conference dinner provided networking opportunities, and brought the community together to work towards the common goals of the summit.

The recommendations from the session participants concerning measurement and standards needs for the various application areas formed the basis for this report.

The agenda for GSRS16 is available at https://www.fda.gov/downloads/AboutFDA/CentersOffices/OC/OfficeofScientificandMedicalPrograms/NCTR/WhatWeDo/UCM515514.pdf .

5.0 GSRS16 Committees and Attendance

5.1 Co-Chairs

Anil Patri, Ph.D., Food and Drug Administration (FDA), US
Paul Howard, Ph.D., FDA, US

[1] http://nanostandards.ansi.org/tiki-index.php

5.2 Organizing Committee

Wim De Jong, Ph.D., National Institute for Public Health and the Environment (RIVM), Netherlands, and Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), European Union (EU)
Fergal Donnelly, M.D., European Commission (EC), EU
Piotr Grodzinski, Ph.D., National Institutes of Health/National Cancer Institute (NIH/NCI), US
Vincent Hackley, Ph.D., National Institute of Standards and Technology (NIST), US
Paul Howard, Ph.D., FDA, US
Wenlei Jiang, Ph.D., FDA, US
Debra Kaiser, Sc.D., NIST, US
Georgios Katalagarianakis, Ph.D., EC, EU
Anil Patri, Ph.D., FDA, US
Ruben Pita, Pharm.D., LL.M., European Medicines Agency (EMA), EU
Reinhilde Schoonjans, Ph.D., European Food Safety Authority (EFSA), EU
Kumiko Sakai-Kato, Ph.D., National Institute of Health Sciences (NIHS), Ministry of Health, Labour and Welfare (MHLW), Japan
Birgit Sokull-Klüttgen, Ph.D., EC–Joint Research Centre (JRC), EU
Katherine Tyner, Ph.D., FDA, US

5.3 Attendance

The meeting was very successful with over 200 attendees from government agencies, industry, and academia from 19 countries. There were about 50 presentations by invited speakers, six panel discussion sessions and over 40 poster presentations by scientists and students on the latest research related to the session topics. Attendees registered at a website for the conference(https://www.fda.gov/AboutFDA/CentersOffices/OC/OfficeofScientificandMedicalPrograms/NCTR/WhatWeDo/ucm488022.htm) and registration was only restricted by total seating in Natcher Auditorium.

Future activities include the development of a publication, and work collaboratively with the community stake holders in developing much needed standards identified at GSRS16 that are relevant for medical and consumer products. There was discussion and suggestions regarding future meetings of a similar vein to maintain the momentum generated by the pre-GSRS15 and GSRS16 meetings.

6.0 Invited Plenary Presentations

The Organizing Committee invited speakers for the plenary sessions from many regulatory authorities, research institutions and industries to ensure that a wide representation (breadth) and knowledge (depth) was present. The invited speakers for the plenary session on Day 1 were (in alphabetical order by last name):

Gerrit Borchard, Ph.D., Univ. Geneva, Switzerland
Robert Califf, M.D., FDA, US

Charles Clifford, Ph.D., National Physical Laboratory (NPL), UK
Hany Demian, M.S., FDA, US
Fergal Donnelly, M.D., EC, EU
Vincent Hackley, Ph.D., NIST, US
Xing-Jie Liang, Ph.D., National Center for Nanoscience and Technology (NCNST), China
Julia Maier, Ph.D., European Pharmacopoeia (EuP), EU
Anil Patri, Ph.D. (FDA, US)
Ruben Pita, Pharm.D., LL.M. (EMA, EU)
Alan Rawle, Ph.D., Malvern Instruments, US
Kumiko Sakai-Kato, Ph.D., NIHS, Japan
Reinhilde Schoonjans, Ph.D., EFSA, EU
Birgit Sokull-Klüttgen, Ph.D., EC–JRC, EU
Kahkashan Zaidi, Ph.D., U.S. Pharmacopoeia (USP), US

To open GSRS16, Dr. William Slikker welcomed the attendees and outlined the genesis of the Global Coalition for Regulatory Science Research (GCRSR) and the Global Summit on Regulatory Science (GSRS) that has brought together regulatory agencies from around the globe as a forum for advancing regulatory science, food safety, medical technologies, and public health. He then invited Dr. Robert Califf (Commissioner, Food and Drugs, US FDA) to present opening remarks and a plenary address for the summit. Dr. Califf outlined the role of FDA in protecting public health through science-based regulation and policy and the importance of regulatory science in that context. For advanced technologies, including nanotechnologies, he highlighted the exciting new developments that will enable personalized and precision medicines. He identified three challenges: (1) Characterization of nanomaterials due to their inherent complexity; (2) Reproducibility of scientific and published data; and (3) Perception of regulatory hurdles. He highlighted the importance of developing appropriate documentary standards and RMs and encouraged attendees to address these challenges collaboratively.

Two plenary sessions followed Dr. Califf's address with the goal of informing attendees about efforts at various agencies around the globe on the current state of nanomaterial use, progress in regulatory science research, and regulatory guidance and standards development.

6.1 International Regulatory Science and Standards Perspectives on Nanotechnology

Co-Chairs: Dr. Anil Patri and Dr. Paul Howard

This session focused on perspectives from government agencies in the US, Europe, and Asia on the current state of nanomaterial knowledge, use, progress, and challenges.

6.1.1 Speakers' Presentations

Dr. Anil Patri started the session by summarizing FDA's experience with reviewing and approving medical products containing different kinds of nanoscale materials. The number and complexity of these products has increased over the years; FDA's understanding of these technologies has also increased through internal regulatory science research, establishment of core facilities with advanced equipment, and reviewer training. He described the establishment

of the Nanotechnology Task Force at FDA in 2006 that helped identify knowledge and policy gaps and enabled the development of many guidance documents pertinent to nanotechnology. FDA staff also participated in coordinating activities with other agencies in the US through the National Nanotechnology Initiative, engaged with stakeholders through Standards Development Organizations (SDOs), and organized workshops to inform industry and the public about responsible development of nanotechnology. Dr. Patri described existing challenges and outlined the scope of the Summit designed to generate a prioritized list of standards immediately for support of regulatory review. Dr. Fergal Donnelly presented a talk on the European Commission's approach to regulatory science for medical technologies, including nanotechnology. He described the enormous costs associated with healthcare product development through the product approval process, and highlighted the importance of better regulations that take into consideration adaptive clinical testing strategies to include information generated prior to testing in humans. The "valley of death" between preclinical, early and late phase clinical trials can be broken down by integrating information such as chemical and physical characterization of products, population-based healthcare data, and biomarker characterization to develop a more complete picture of how the human body performs and responds in healthy and diseased states. A lifecycle management approach to products that takes account of the continual evolution of science is needed for maximal protection of the public. Dr. Ruben Pita gave a presentation on the framework for medicines in the EU, interactions between various agencies in the EU member states, and a regulatory overview of nanomedicines in the EU. He described the importance of standardization and harmonization to maximize the benefits of nanotechnology, EMA initiatives in collaborative standards development, and the many reflection papers the EMA has published. Dr. Reinhilde Schoonjans presented a talk on the increased use of nanomaterials in agri/food/feed applications that are subjected to scientific risk evaluation in Europe and EFSA's risk assessment strategies. She outlined the challenges in addressing human health risk assessment and the lack of data and test methods available to conduct a thorough assessment. EFSA has identified four areas of investment: (1) advanced methods for physico-chemical characterization of nanomaterials including in complex matrices; (2) absorption, distribution, metabolism, and excretion (ADME) studies; (3) toxicology studies; and (4) instilment of new legal requirements to assist stakeholders and risk assessors in the EU Member States prepare authorization dossiers for protection of consumer safety. Dr. Kumiko Sakai-Kato gave a presentation on Japan's regulatory science and standards perspective and highlighted the importance of product quality, efficacy, and safety of medical products. Dr. Kumiko mentioned that there are no new regulations in Japan specifically designed for nanotechnology-based drug products and that such drugs are evaluated on a product-by-product basis. She also outlined the guidance and reflection papers that have been issued from Japan and the country's participation in standards development activities. The session concluded with a presentation from Dr. Xing-Jie Liang on the advancement of many drug products containing nanomaterials into clinical trials in China and the importance of understanding how physico-chemical attributes impact biological effects, product quality, safety and ADME and toxicity (ADMET). Dr. Liang discussed the

challenges in drawing conclusions from inconsistent results obtained by different laboratories and the need for interlaboratory studies towards establishing standards.

6.2 International Standards Perspectives on Nanotechnology

Co-Chairs: Dr. Debra Kaiser and Dr. Birgit Sokull-Klüttgen

This session focused on providing the Summit attendees an overview of the standards development processes at various organizations, existing standards, and pathways for the development of new standards. The objective was to enable informed discussions during the breakout sessions aimed at generating prioritized lists of needed standards. Speakers were from various SDOs and national metrology institutes.

6.2.1 Speakers' Presentations

Dr. Vincent Hackley outlined the importance of RMs, *i.e.*, physical standards with well-defined values for one or more properties, and described the development and applications of nanoscale RMs using a case study on colloidal gold RMs developed at NIST. These standards have aided regulatory and preclinical science studies by enabling method validation, interlaboratory comparison studies, measurement controls, instrument calibration or qualification, and performance testing. They have been used globally across a range of applications, and serve as enablers of measurement science and standardization. Dr. Birgit Sokull-Klüttgen delivered a presentation on the differences between RMs, certified RMs (CRMs), and the use of 'representative test materials' (RTMs). RTMs are used to fill a gap due to the lack of appropriate RMs in the field of nanotechnology. Based on scientific and testing community needs for RTMs, the JRC acquired industrial nanomaterials and established the JRC Nanomaterials Repository. These materials were used in the Organization for Economic Cooperation and Development (OECD) testing program and were distributed to various EU-funded projects, the results of which contributed to the characterization of these materials. Dr. Gerrit Borchard presented on the clinical differences observed in different iron sucrose products and outlined the development and validation of size measurement methods for these products using dynamic light scattering. Dr. Alan Rawle's presentation focused on ASTM International Committee E56 on Nanotechnology. He described the scope of the committee, the various subcommittees within E56, and the various test methods and guides that were developed in the areas of characterization, health, safety, and work force training. Dr. Charles Clifford presented an overview of ISO standardization with specific emphasis on ISO Technical Committee (TC)229 on Nanotechnologies. He outlined various subcommittees in terminology, measurement and characterization, health, safety, and the environment, and material specifications. Dr. Clifford also highlighted the linkages of TC229 to other relevant ISO committees including surface chemical analysis and particle characterization. Dr. Kahkashan Zaidi gave a presentation on USP's standards-setting structure and processes for the identity, strength, quality, and purity of medicines, food ingredients, and dietary supplements manufactured worldwide. USP interacts closely with FDA (US) and other government agencies in setting standards through Expert Committees and meetings. Mr. Hany Demian described the benefits of development and use of standards and outlined FDA's involvement in this process

along with stakeholder involvement through SDOs. He presented the perspectives of standards use from various Centers within FDA and mentioned that over 700 FDA representatives participate in over 1000 active committees in various SDOs.

7.0 Parallel Breakout Sessions

Breakout sessions were held on the six topics listed below. The sessions consisted of invited presentations and a panel discussion with audience participation. The last hour of each session was an open brainstorming discussion on the following topics:

- Needs for advances in regulatory science, instrumentation and methods
- Relevance and applicability of existing standards and adoption by industry
- Needs for new standards to facilitate regulatory review

For each session, an independent report was prepared that included a short introduction of the field, a brief summary of the presentations, and a summary of the panel discussions with three sections: (1) Documentary standards of interest; (2) Challenges and considerations; and (3) Prioritized regulatory science research and standards needs. There were two parallel, concurrent sessions in the morning and afternoon of Day 2 and the morning of Day 3.

The invited speakers for each breakout session are listed below.

Advances in Nanotechnology-Derived Drug Products (Section 7.1)

Katherine Tyner, Ph.D., FDA, US
Ruben Pita, Pharm.D., LL.M., EMA, EU
Piotr Grodzinski, Ph.D., NIH/NCI, US
Neil Desai, Ph.D., Celgene, US
Lawrence Tamarkin, Ph.D., Cytimmune, US
Xiaoming Xu, Ph.D., FDA, US
Kenneth Dawson, Ph.D., Univ. College Dublin, Ireland – *moderator*
Vincent Hackley, Ph.D., NIST, US – *rapporteur*

Advances in Nanotechnology-Derived Medical Devices (Section 7.2)

Peter Goering, Ph.D., FDA, US
Indira Hewlett, Ph.D., FDA, US
Wim De Jong, Ph.D., RIVM, Netherlands and SCENIHR, EU
Hari Sharma, Ph.D., Uppsala Univ., Sweden
Liming Xie, Ph.D., NCNST, China
Brendan Casey, Ph.D., FDA, US – *moderator*
Rosalie Elespuru, Ph.D., FDA, US – *rapporteur*

Liposomal Drug Products (Section 7.3)

Frank Szoka, Ph.D., Univ. California San Francisco, US
Kumiko Sakai-Kato, Ph.D., NIHS, Japan
Esther Chang, M.D., Georgetown Univ., US

Stephen Stern, Ph.D., NCI/Nanotechnology Characterization Laboratory (NCL), US
Diane Burgess, Ph.D., Univ. Connecticut, US
Duanyun Si, Ph.D., NCNST, China
Wenlei Jiang, Ph.D., FDA, US – *moderator*
Ruben Pita, Pharm.D., LL.M., EMA, EU – *rapporteur*

Nanomaterials in Food and Food Contact Materials (Section 7.4)
Wim De Jong, Ph.D., RIVM, Netherlands and SCENIHR, EU
Chia-Ding Liao, Ph.D., TFDA, Taiwan, Republic of China
David Lefebrve, Ph.D., Health Canada, Canada
Timothy Duncan, Ph.D., FDA, US
Treye Thomas, Ph.D., Consumer Product Safety Commission (CPSC), US
Albert Braeuning, Ph.D., German Federal Institute for Risk Assessment, Germany
Sangeeta Khare, Ph.D., FDA, US
Reinhilde Schoonjans, Ph.D., EFSA, EU – *moderator*
Dragan Momcilovic, Ph.D., FDA, US – *rapporteur*

Targeted Nanomaterials for Biomedical Applications (Section 7.5)
Kenneth Dawson, Ph.D., Univ. College Dublin, Ireland
Rangaramanujan Kannan, Ph.D., Johns Hopkins Univ., US
Lily Yang, Ph.D., Emory Univ., US
Marina Dobrovolskaia, Ph.D., NCI/NCL, US
Clarice Hutchens, Ph.D., Pfizer, US
Jan Simak, Ph.D., FDA, US
Piotr Grodzinski, Ph.D., NIH/NCI, US – *moderator*
Wimolnut Manheng, Ph.D., FDA, US – *rapporteur*

Nanomaterials in Personal Care Products (Section 7.6)
Nakissa Sadrieh, Ph.D., FDA, US
Birgit Sokull-Klüttgen, Ph.D., EC–JRC, EU
David Andrews, Ph.D., Environmental Working Group, US
Monita Sharma, Ph.D., PETA International Science, England
Shou-Chieh Huang, Ph.D., Taiwan Food and Drug Administration (TFDA), Republic of China
Sri Nadadur, Ph.D., NIH/National Institute of Environmental Health Sciences (NIEHS), US
Nigel Walker, Ph.D., NIH/NIEHS, US – *moderator*
Paul Howard, Ph.D., FDA, US – *rapporteur*

7.1. Advances in Nanotechnology-Derived Drug Products

Co-chairs: Dr. Katherine Tyner and Dr. Ruben Pita

7.1.1. Introduction

In recent years, there has been an increased focus on developing drug products containing nanomaterials. With this increased focus, there has been a corresponding increase in

applications for drug products containing nanomaterials submitted for regulatory review. Nanomaterials can be present in drug products to perform different functions, including serving as active pharmaceutical ingredients or as formulation excipients (including as carriers loaded with an active ingredient). Although subject to the same rigorous regulatory standards as any other drug product, unique properties that arise from the structural and functional complexity of nanomaterials may lead to additional scientific considerations when following current guidelines and practices. Defining a suitable battery of tests for the characterization, in-process controls and quality controls of drug substances, excipients, and finished products is critical to guarantee that the safety and efficacy of medicines are reproducible from their development to post-marketing lifecycle management. In the fast evolving and complex environment of nanotechnology, determining the necessary tests and methods can be challenging. Collaborations between regulators, standardization bodies, industry, and academia will promote the translation of drug products containing nanomaterials from research to the patient.

7.1.2 Speakers' Presentations

Dr. Katherine Tyner kicked off the session by highlighting FDA's priorities for nanotechnology documentary standards and RMs that could be used in support of drug products containing nanomaterials. Specifically mentioned was the alignment with the most common analytical techniques (*e.g.* dynamic light scattering, high resolution microscopy) seen to date by the Agency, size RMs (1-300 nm), and carbon-based RMs. Dr. Ruben Pita emphasized the continuous collaboration between regulatory authorities from different regions to promote the standardization of regulatory requirements and underlined the need to correlate the priority setting of nanotechnology standards with the most relevant critical quality attributes (CQAs). Dr. Piotr Grodzinski discussed the status of nanotechnology used in efforts funded by the Alliance for Nanotechnology in Cancer and described future opportunities and strategies in this field. Examples included new modes of therapy including small interfering RNAs (siRNAs) and kinase inhibitors and new imaging techniques based on nanoparticles designed to operate in a multi-functional manner. Dr. Grodzinski also stressed that access to reliable good laboratory practice (GLP) characterization and good manufacturing practice (GMP) facilities will need to become more available to advance the field. Dr. Neil Desai presented research on an albumin nanoparticle-based drug delivery platform that utilizes the transport and binding properties of albumin to achieve enhanced tumor penetration and accumulation of hydrophobic drugs while eliminating the need for toxic solvents. Dr. Desai also discussed the complexity of characterizing this type of delivery system and the adequacy of designing comparative studies between products proposed to be equivalent. Dr. Lawrence Tamarkin focused on drug products containing nanomaterials for targeting cancer, the importance of understanding the biology of the tumor in the development of new products, and on a specific formulation development matrix used to streamline the optimization of final drug products. Dr. Xiaoming Xu concentrated on the unique physicochemical properties of nanomaterials, such as small size, large surface area to mass ratio, high reactivity, and varied *in vivo* pharmacokinetics (PK) characteristics, and

discussed the common risk factors and quality attributes associated with nanomaterial containing drug products.

7.1.3. Panel Discussion

The main topics from the panel discussions are summarized in the following sections.

7.1.3.1. Documentary standards of interest

The following topics were identified as being of interest for documentary standards development.

- *In vitro* release and stability testing, including stress testing
- Surface characterization (including quantification and composition conformation of surface ligands); RMs are also of interest
- Analytical techniques used to evaluate the most common quality attributes seen for drug products containing nanomaterials
- Reporting and data analysis of analytical techniques used in characterization studies;
- Bioequivalence measurements for drug products containing nanomaterials, including typical measurands such as AUC (area under the plasma concentration time curve) or C_{max} (peak drug concentration)
- Methodologies to link physico-chemical measurements with clinical relevance

7.1.3.2. Challenges and considerations

Overall, there is great diversity of opinion and lack of understanding from potential end users (*e.g.* developers of drug products) of what standards are and how they are used during scientific development and regulatory assessment. This general uncertainty is compounded by the frequently complex nature of drug products containing nanomaterials, which makes standardization challenging. In addition, there is great diversity across the landscape of drug products containing nanomaterials, including the specific types of nanomaterials used and how they are being used within the drug product. The expectation of “one size fits all” for standards development is not realistic, nor is the product-by-product development of standards. Thus, there is a desire to balance the need for standards with the utility and/or applicability of those standards to a product class.

The need for standards is valid for the entire lifecycle of the product (development to preclinical to generics to post-marketing analysis), and there is a general need for understanding how to assess equivalency of methods and validity of methods for their intended purpose and how to ensure that the measurements are statistically relevant. RMs are a critical component for this work, as is robust partnership between industry, regulators and standards developing organizations.

7.1.3.3. Prioritized regulatory science research and standards needs

The presentations and panel discussions concluded with the following points.

7.1.3.3.1. Outreach

Two key action items agreed upon by the session participants were to promote awareness of the need for use of standards, even in early drug development, and to have access to documentary standards (or guidance/regulatory notes) available for methods and standards validation.

7.1.3.3.2. Research, relevance, and prioritization

FDA, EMA and JRC have collected the CQAs most frequently seen in regulatory submissions for drug products containing nanomaterials. There is general agreement to analyze and rank the CQAs to form a prioritized list of needed documentary standards. A logical forum for this work is the International Pharmaceutical Regulators Forum Nanotechnology Working Group (https://www.i-p-r-f.org/en/working-groups/nanomedicines-working-group/). Related to this effort is the desire for research on the clinical relevance of the top ranked CQAs, including *in vitro* and *in vivo* correlation and impact of extent of change of a CQA.

7.1.3.3.3 Priority standards

Session participants identified the following standards as being of highest priority.

Documentary standards

- Methods for the top ranked CQAs (including standard practices or standard test methods)
- General considerations for drug products containing nanomaterials (*e.g.*, a standards guide, decision trees for methods to use)
- Data analysis and reporting

Reference materials

- Liposomes for size and composition certification
- Particulate materials with average particle sizes between 1 nm and 300 nm
- Surface functionalization relevant to medical applications

7.2. Advances in Nanotechnology-Derived Medical Devices

Co-Chairs: Dr. Peter Goering and Dr. Wim De Jong

7.2.1. Introduction

Nanotechnology is expected to critically impact the design, development, and manufacture of next-generation medical devices. Examples of nanomaterial-enabled devices include bone scaffolds, dental filler materials, wound dressings and catheters with antimicrobial coatings, *in vitro* diagnostic kits for pathogens and cancer biomarkers, and imaging contrast agents. Further, the functionalization or modification of medical device surfaces with nanotechnology, modulating the chemistry to introduce preferred nanoscale topographies, or physically etching the surface to create nanoscale features are approaches being considered to provide enhanced cell proliferation and tissue integration. Nanomaterials offer size-attributed properties, such as large surface area and catalytic and anti-microbial activity, making them attractive candidates for use in the medical device industry. Along with these technological advances, broad

questions have been raised related to physico-chemical characterization, consistency in manufacturing and quality, and safety. Scientists from the diverse sectors of industry, academia and government are working together to address these issues and to develop standards appropriate to the use of nanomaterials for medical devices. There are several notable standards, technical reports, and guides that can facilitate the safety assessments of nano-enabled medical devices listed in Table 1 at end of this report.

7.2.2 Speakers' Presentations

The session on medical devices featured five presentations illustrative of the diversity of nanomaterial use in medical device materials and diagnostic assessments. Dr. Peter Goering addressed the safety and efficacy evaluation of immobilized surface nanostructures. Dr. Indira Hewlett discussed the use of nanotechnology approaches for pathogen detection using *in vitro* diagnostic assays. Dr. Wim De Jong addressed the risk assessment of nanomaterials and the application of standards for medical devices. Dr. Hari Sharma discussed the use of functionalized gold and magnetic iron oxide nanoparticles in the context of medical practice, particularly neurological diseases. Dr. Liming Xie presented the need for physico-chemical characterization and biological assessment of nano-silver and the protocols developed by NCNST for nano-silver toxicity evaluation.

Dr. Peter Goering noted that one of the key values of standards are the contributions to speeding up the FDA review process, because of the guidelines provided to sponsors and the common understanding between FDA and industry. FDA's Center for Devices and Radiological Health (CDRH) has a standards recognition process but only a few nanomaterial standards have been recognized by FDA/CDRH thus far. As noted in the presentations, the use of nanomaterials in medical devices includes: (1) products used for therapy such as wound dressings; (2) implanted devices nanoscale surface topography, *e.g.,* orthopedic and cardiovascular devices; (3) drug delivery systems; and (4) products used for diagnostic tests. The concern for diagnostic products using nanomaterials is in quality and reproducibility of the tests, whereas the therapeutic and implant products have an additional safety context.

7.2.3. Panel Discussion

A roundtable discussion was held with the speakers that included audience participation via comments and questions. The discussion centered on the following topics.

7.2.3.1. Documentary standards of interest

Many existing standards, technical reports, and guides can be used for nanomaterials, *e.g*., the ISO/TC 229 and ASTM International E56 technical documents for physical characterization of nanomaterials. Safety evaluation and requirements for nanomaterials were initially limited to air pollutants (*e.g*., diesel exhaust); however, other uses such as applications for drugs and medical devices are emerging; we need to re-think the toxicology assessment of nanomaterials. The ISO 10993 series of standards for medical devices can be used for assessment of nanomaterials and is an important resource. An overview of nanomaterial assessment is provided in ISO 10993-22, which will be published in 2017.

The ISO 10993-22 document provides insight and knowledge on how to deal with nanomaterials applied in medical devices. For each of the considerations in biological safety testing of devices with nanotechnology, nano-specific issues are discussed, including the impact of physico-chemical properties on safety and efficacy and the need for proper physico-chemical characterization. Common strategies and various pitfalls of biological safety evaluation of nanomaterials are also discussed.

Standards, technical reports, and guidelines for biological assessment of nanomaterials associated with medical devices are certainly needed. For practicality, the safety of devices should be considered first in standard systems; then the assessment could move into models of disease states or targeted human diseases for examination of nanomaterial safety and efficacy in a certain context (see below).

7.2.3.2. Challenges and considerations

Existing, common assays for material safety assessment are generally applicable, but some may need modification for nanomaterials. For instance, the current standards for genotoxicity assessment of nanomaterials are not sufficient. The standard bacterial assays are problematic because of the lack of uptake of nanomaterials, while some genotoxicity assays using mammalian cells utilize reagents that inhibit uptake (*e.g*., cytochalasin B in the *in vitro* micronucleus assay). Common issues with some assays include interference with signal output due to, *e.g*., inherent nanomaterial fluorescence and light scattering in spectrometry read outs. In addition, the potential for sequestration or accumulation of nanomaterials in certain tissues or organs need to be considered. The extreme diversity of nanomaterials causes difficulty in generalizing approaches.

A major challenge for safety assessment concerns the use of pristine nanomaterials or nanomaterials in manufactured products. There was a diversity in participant opinion in response to this issue. Some assessments choose pristine nanomaterials for *in vitro* and *in vivo* tests while others test nanomaterials as found in the finished medical device products. Evaluation of the toxicity of pristine nanomaterials offers the possibility to select “less” toxic nanomaterials as starting materials for use in medical devices. It should be noted that in most cases, regulatory bodies require testing on final, finished, sterilized medical devices as opposed to individual components, such as nanomaterials used in manufacturing the device.

Challenges for *in vitro* diagnostics using nanomaterials include variations in starting materials (*e.g*., consistency and quality in production over time), acceptance criteria for materials, variations in functionalization, and the combination of physical and functional testing. For *in vitro* diagnostics, there is a need for RMs of nanomaterials that are used repeatedly, *e.g*., silver (NIST RMs), gold (NIST RMs), and silica (JRC RMs). There is a need for reference methods and materials for both lab-based and point of care (POC) assays.

One consideration is whether it is possible to categorize nanoparticles for creating standards that will be applicable to a broader class of nanomaterials. In the context of categorization, it was agreed that nanomaterial size constitutes an important “horizontal” category. Smaller

nanoparticles also tend to be more toxic compared to larger nanoparticles for a variety of reasons, including higher capacity for cell uptake, increased ability to reach various organs after absorption, and larger surface area resulting in greater surface energy or dissolution of potential toxic ions. For example, TiO_2 and ZnO used as ultraviolet (UV) filters in sunscreen were found to group into risk categories based on particle size and catalytic activity. In an EU analysis, 17 manufacturers provided toxicity data (*e.g.* for TiO_2 http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_136.pdf). This may provide a guideline for new products if they fit the boundaries of this analysis; however, size alone is not sufficient to determine the safety or effectiveness of a nanomaterial. There might be other criteria for a different "horizontal" categorization relative to function of nanomaterials within a given use context. It was also suggested that studies be conducted on commonly used nanomaterials, *e.g.*, TiO_2, Ag, Au, Si. These studies would constitute valuable "vertical assessments" of these nanomaterials. It is important to determine whether a given nanomaterial is released from a product resulting in patient exposure; this is critical to enable the assessment of health risks involved.

In summary, the fundamental issues for all nanomaterial assessments include: (1) both physico-chemical and functional/biological testing; and (2) extrapolating results from test systems to humans.

7.2.3.3. Prioritized regulatory science research and standards needs

Currently, the overwhelming majority of nanomaterial standards, technical reports, and guidelines are focused on physico-chemical characterization. Such technical documents are crucial to ensuring the proper nanomaterial characterization to ensure experimental data can be correlated and compared. They are essential to determine consistency and quality in the production of nanomaterials. In contrast, there is a paucity of standards detailing proper techniques and protocols for biocompatibility assessment of nanomaterials. The group has identified this as a prioritized need; however, it may be possible to adapt current standards for nanomaterials, *e.g.*, adaptations of a hemolysis assay (ASTM E2524), to accommodate nanomaterial testing. Lastly, key standards, technical reports, and guides identified in Table 1 should be considered for recognition by regulatory agencies.

The following tiered approach for prioritized standards development was recommended as broadly benefiting consumer and biomedical stakeholders.

> **Tier 1 (Highest):** *Standards broadly addressing general considerations of nanomaterials.* Such standard(s) would provide an overview of material properties unique to nanomaterials, suggested testing, and common testing pitfalls. Such standard(s) would provide a foundation for nanomaterial standards that is desired or necessary, but would not provide specific testing protocols (*e.g*, ISO 10993-22).
>
> **Tier 2:** *Horizontal standards that could be applied to a group of nanomaterials such as metal oxide nanoparticles, e.g., for assessing size and shape using electron microscopy.*

These standards would provide great value given their applicability to a broad "category" of nanomaterials.

Tier 3: *Vertical standards that are primarily relevant for one specific type of nanomaterial, e.g., gold nanoparticles.*
These standards would discuss the specific protocols/techniques that could be used for physico-chemical characterization and biological assessment for a specific nanomaterial.

Tier 4 (Lowest): *Standards that provide a specific technique for a specific nanomaterial (e.g., atomic force microscopy of gold nanoparticles).*
Although such standards may be valuable for highly utilized materials, *e.g.,* gold or silver nanoparticles, the high degree of specificity is of less value than the more broadly applicable Tier 1-3 standards.

7.3. Liposomal Drug Products

Co-chairs: Dr. Wenlei Jiang and Dr. Kumiko Sakai-Kato

7.3.1 Introduction

Since their discovery in the 1960s [1], the use of liposomes in the medicinal field has been greatly explored by pharmaceutical scientists [2]. Many liposomal drug products are designed to maximize therapeutic effects and minimize adverse effects by enhancing the *in vivo* stability of the active substance, its biodistribution to target tissues and organs, and, in some cases, its intracellular trafficking.

One of the major challenges in the development and evaluation of liposomal drug products is identification of physicochemical properties that affect the *in vivo* properties of the drug product and development of suitable analytical techniques to assess both *in vitro* and *in vivo* properties. Some regulatory agencies have issued general or product-specific liposome product guidance to discuss critical properties of liposomes and to guide pharmaceutical scientists in liposome product development [3].

In the session of liposomal drug products, speakers from academic institutions and government research agencies described the state of science and regulatory requirements in liposome drug products, and prioritized regulatory science research and standards needs.

7.3.2 Speakers' Presentations

Dr. Frank Szoka gave a presentation on the development and validation of a new *in vitro* liposome doxorubicin release assay. The assay promotes drug release from liposome doxorubicin formulations by either physical or chemical stress, in a manner that may enable the determination of the degree of similarity between the innovator and generic products. Dr. Stephan Stern focused his presentation on pharmacokinetics and drug release, addressing the importance and challenges of monitoring the dispositions and *in vivo* integrity of nanotechnology platforms, including liposomal formulations, highlighting potential problems with current bioanalytical techniques. He also introduced a stable isotope tracer methodology currently under evaluation. Dr. Duanyun Si presented on *in vivo* PK/ pharmacodynamics (PD)

assessments, and emphasized the importance of *in vivo*-based pharmacology and toxicology studies as well as PK studies for evaluation of the performance of liposomes. Dr. Kumiko Sakai-Kato gave an overview of the Japanese liposome guideline. She also presented the regulatory research activity at NIHS, and introduced the development of analytical methods for liposome drug products. Dr. Esther Chang reported the clinical trial progress using cationic liposomes decorated with single chain antibody fragments for active targeting. Dr. Diane Burgess presented her recent research on the development of a novel continuous manufacturing method for liposomal formulation and the properties of the liposomes produced.

7.3.3. Panel Discussions

7.3.3.1. Documentary standards of interest

Based on the presentations above, the speakers discussed the regulatory science research and standards needs for liposomal drug products in terms of lipid excipients, liposomal drug products, and *in vivo* test methods.

7.3.3.1.1. Formulation composition

Liposomes mainly consist of one or more active pharmaceutical ingredients (API) and lipid excipients. Session participants felt that many RMs are available for small molecule API but not necessarily for nucleic acids or other macromolecules; some CRMs are available from JRC for Clinical, Health, Food and Feed related applications (https://crm.jrc.ec.europa.eu/). Lipids are critical excipients in liposome formulations. Participants shared their experiences of using lipids and commented that lipid quality from different manufacturers is generally well controlled. In addition, some monographs for RMs are available for lipids. Participants proposed that standards for additional lipid excipients should be developed and RMs for commonly used lipids are needed.

7.3.3.1.2. Product characterization

A consensus was reached that the current panel of tests are generally sufficient to characterize conventional liposome formulations; however, additional characterization is needed for future generations of liposomes, *e.g.*, targeted liposomes. Particle size and surface properties are critical aspects of liposome formulations. Standard procedures and RMs are available for particle size measurements. Standard procedures for zeta potential measurement were also developed but different instrument companies provide different RMs for zeta potential measurement. Session participants recommended development of standardized RMs for zeta potential measurement. Additional needs for liposome product characterization were noted: particle concentration measurements; particle polydispersity index determinations through fractionation methods; recommendations about *in vitro* active pharmaceutical ingredient (API) release testing procedures, and standard procedures for endotoxin testing and complement activation. The potential need for a reference liposome (drug-empty) material that can aid in the examination of stress during the production characterization process was also noted.

7.3.3.1.3. *In vivo* pharmacokinetics

As discussed by speakers in their presentations, some separation procedures of free and liposomal encapsulated drugs in biosamples may introduce artifacts in measurements. Participants agreed with the need for standardized or improved procedures for free and liposomal drug separation, or the development of bioanalytical methods that can measure free and liposomal encapsulated drug simultaneously.

With regard to liposomal drug development, guidance documents have been published by FDA [3], MHLW [4], and EMA [5]. To the question of whether the current liposome guidance helps in early development and life cycle management of liposome formulations, participants generally showed a positive response.

7.3.3.1.4. General considerations for liposome product development

It was agreed that Quality-by-Design (QbD) and design-of-experiment approaches are recommended in liposome product development geared to optimize the CQAs. QbD will allow development of better understood and characterized products and will help develop meaningful standards for excipient, liposome product, and process control.

7.3.3.2. Prioritized regulatory science research and standards needs

The prioritized regulatory science research and standards needs were summarized as follows:

- RMs for commonly used lipids in liposome formulations
- RMs for zeta potential and surface component measurement of liposome formulations;
- Standardized or improved procedures for the free and liposomal drug separation or simultaneous quantification of liposomal encapsulated and free drug

7.4. Nanomaterials in Food and Food Contact Materials

Co-chairs: Dr. Reinhilde Schoonjans and Dr. Dragan Momcilovic

7.4.1. Introduction

This session focused on nanomaterials in agriculture/food/feed products, including food contact materials. The application of nanoscience has been widely predicted to have a major impact on the current trends, including materials and technologies employed in the agriculture and the food/feed chain. However, so far, not many applications of nanotechnology have been marketed by the food-related sectors in the EU, which can be explained in part by the stringent EU regulatory safety requirements. In other parts of the world, where such requirements for nanotechnology products may be relatively relaxed, or not in place, several products are already available on the market. Examples include: (1) both inorganic nutrients and supplement additives, such as calcium, magnesium, selenium, iron, zinc, silver, gold; and (2) organic additives/supplements, such as some vitamins, isoflavones, ß-carotene, lycopene, lutein, omega-3 fatty acids, coenzyme-Q_{10}, benzoic acid, citric acid, ascorbic acid, and curcumin. In most cases, the use of nano-sized nutrients and supplements in the body is intended (or claimed) to enhance uptake, absorption, and bioavailability compared to bulk equivalents [6].

In the EU and US, nanomaterials must undergo a scientific risk assessment to evaluate if the proposed use levels in the food/feed chain are safe for consumers, animals, and the environment. To perform the required safety studies, it is desirable to have available standards that are accepted by international regulatory bodies. One of the key aspects is that the safety study should be performed with the food grade form of the nanomaterial and at levels that are physiologically relevant. This requires a proper physicochemical characterization at the time the material is sampled for testing (pristine and as existing in the test medium) and proper detection mechanisms after the administration of the material to the test system (*in vitro* or *in vivo*). Many organizations have already invested in the development of documentary standards (*e.g.*, ISO, ASTM International, the European Committee for Standardization (CEN)); nanoscale RMs, (*e.g.,* metrology institutes in the US, EU, Japan, Germany, Canada); guidance documents (*e.g.*, OECD and agencies in Australia, the EU, Japan, the US); and publicly available protocols (*e.g.,* NCI/NCL and NIST).

7.4.2 Speakers' Presentations

Six speakers from Asia, the EU, and North America delivered presentations on diverse nanoproducts in the pipeline or on the market and current insights regarding safe use of the products, *e.g*., detection, tissue distribution, accumulation, and effects on the microbiome. Dr. Wim De Jong presented two risk assessment approaches for synthetic amorphous silica (SAS) and TiO_2: one based on oral intake (external exposure) and no-observed-adverse-effect-level (NOAEL); and another based on internal organ concentration using kinetic modelling. Despite the uncertainties and assumptions in the studies, risk could not be excluded. Dr. Chia-Ding Liao described the range of results from physicochemical characterization on different batches of the popular pearl-powder supplement in Taiwan and TiO_2 added to whiten chewing gum. He also explained that in Taiwan, milk has been regularly supplemented with nano-forms of calcium. An evaluation of available artificial gastrointestinal digestion and absorption methods was reported by Dr. David Lefebvre. This approach is considered useful for assessing the stability and quantifying absorption of engineered nanomaterials. Furthermore, he showed research results for the immunotoxicity of carbon black nanoparticles that modified the expression of allergy-associated Th2 markers in the spleen. Regarding potential consumer exposure to food contact materials containing nanomaterials, Dr. Timothy Duncan discussed work on model systems to link physicochemical properties to oral and environmental exposure kinetics. On the topic of migration, Dr. Treye Thomas explained that CPSC-FDA interagency research confirmed that current FDA guidance for evaluating migration of food contact materials into food is applicable to nanomaterial migration. Findings on the uptake and transport of orally ingested silver nanoparticles (Ag-NPs) were presented by Dr. Albert Braeuning. Ag-NPs reach the gastrointestinal epithelial cells, but only a small quantity pass the intestinal barrier to become systematically bioavailable. The consequences of long-term intake on the human body remain to be elucidated and new modelling techniques as well as relevant endpoints were discussed. Dr. Braeuning also pointed out the challenges in differentiating ionic silver and nano-form silver on internalization. The effects of silver nanoparticles on the

intestinal microbiome was described by Dr. Sangeeta Khare, who reported size-dependent, adverse effects on the commensal microbiota. In addition, he found gut-associated immune responses and the presence of silver resistance genes in bacterial populations within the gastrointestinal tract resulting from exposure to silver nanoparticles.

7.4.3. Panel Discussion

7.4.3.1. Challenges and considerations

The full audience participated in a brainstorming session to map the needs for standards to support regulatory science and actions in this area. One conclusion was that a one-size-fits-all approach will not be possible for all nanomaterials in all situations, as the release in different media/food/feed matrices will influence nanomaterial detection. This session enabled regulators from different parts of the world to share information on how nanoproducts in agriculture/food/feed are assessed and managed. The differences in approaches can be further defined and addressed given more time. It was not explicitly discussed how identified standards needs should be addressed, but it was emphasized that the GSRS should continue to bring standards needs to the attention of the many international organizations that could potentially cooperate in standards development. The panel also discussed the importance of *in vivo* studies and the need for inclusion/quality criteria as guidance for the research community to produce results that are useful for risk assessment. The pros and cons for oral gavage, long-term testing, and different protocols for different age groups were discussed. For example, one concern was the shifting composition of gavage materials in different age groups, and another concern was the lack of involvement of the oral cavity.

7.4.3.2. Prioritized regulatory science research and standards needs

For regulatory science regarding nanomaterials used in agriculture/food/feed products, including food contact materials, the following immediate needs were agreed on and are identified below.

7.4.3.2.1. Priority standards needs for physico-chemical measurements

The prioritization for standards and standard methods focuses on three key needs:

<u>Need 1</u>: Detection of nanomaterials in complex matrices

Needed for collecting data on dietary exposure and detection in body tissues as part of risk assessment, as well as needed for control of products as part of risk management

Focus first on sample preparation protocols:

Preparation of diets (for toxicity studies)

Separation of the nano-forms from the matrix components (for controls)

<u>Need 2</u>: Determination of valence/oxidation states of metals (*e.g.*, Ag, Cr)

<u>Need 3</u>: Precision methods and RMs for measuring:

Numbers of nanoparticles

Weight concentration versus number concentration

7.4.3.2.2. Priority standards needs for safety studies

There are six specific challenges to the need for standards and standard methods for safety studies. These are listed below.

(1) Migration protocols

The specific material needs to be determined (*e.g*., carbon nanotubes);
Case-by-case adjustments depending on the matrix will be needed, but a standard will inform about best practices for at least in one situation

(2) Artificial digestions with food matrix *in vitro*

Could be a "low hanging fruit", as there might be existing options (*e.g.,* standard composition of stomach fluid for GMO testing) that could be adopted as a standard for nanoparticles
Must be complemented with sublingual absorption
How to account for newly formed nanoparticles in gastrointestinal tract

(3) Dosimetry for *in vitro* studies (to increase comparability)

(4) Testing of the microbiome to be further explored (*e.g*. via spot tests?)

(5) Models for tissue distribution and for (oral) absorption

(6) Protocols to determine the effects of coatings on distribution, toxicity, and fate

7.5 Targeted Nanomaterials for Biomedical Applications

Co-chairs: Dr. Piotr Grodzinski and Dr. Xing-Jie Liang

7.5.1. Introduction

This session focused on complex, multifunctional and targeted nanomaterials for biomedical applications. It is expected that nanomaterials containing a targeting agent can be delivered to the intended site of action more efficiently. Speakers from academic institutions, government agencies, and industry described research on such targeted nanomaterials concerning their design, characterization, and potential effects on the immune system.

Several dimensions of the challenges for developing drug products containing targeted nanomaterials were evaluated and discussed. First, the advantages of nanomaterials used as primary vehicles for targeted therapies were discussed. This is possible because nanomaterials can pass biological barriers, enter cells, and then distribute within cells. Speakers presented their preclinical research related to the development of drug products containing nanomaterials that deliver drugs to several specific target organs. Second, most studies have shown that nanomaterial properties, such as size, nature of surface, ligand coating, and colloidal stability can influence how cells internalize nanomaterials, and may contribute different responses to the immune system, which may subsequently induce adverse effects in patients. Therefore, the design and characterization of nanomaterials were considered as key areas of development for drug products containing nanomaterials. Tools are needed for chemical characterization, molecular and functional characterization (*e.g..,* molecular affinity and receptor specificity at the cellular level), biological and pharmacological activities relevant

to clinical application (proof-of-concept), and immunotoxicology tests. Third, the Nanomedicines Alliance consortium involving several biotech and pharma companies was described and discussed. The Nanomedicines Alliance program is a shared resource (*e.g.,* standard methods, publications, scientific meetings) for advancing drug products containing nanomaterials from research through commercialization.

7.5.2 Speakers' Presentations

Dr. Kenneth Dawson introduced the concept of "statistically defined drugs" as a means of better categorizing nanomaterials. This concept has been used as a method to evaluate nanoparticles on a particle-by-particle basis. He also demonstrated a microfluidic device that works like flow cytometry (peptide-ometry) to map the surface of targets and receptors of nanoparticles. Dr. Rangaramanujan Kannan discussed targeted dendrimer nanotherapies for central nervous system (CNS) disorders and eye disease. In these studies, hydroxyl-poly(amidoamine) dendrimers are delivered to the CNS through activated microglia and astrocytes in animals with cerebral palsy. Rabbits' motor functions are significantly improved after a single injection along with decreases in neuroinflammation and oxidative/neuronal injury. Dr. Lily Yang discussed drug delivery of theragnostics for cancer therapy, where targeted constructs are capable of digesting stroma and accessing tumor cells in pancreatic and breast cancers. Dr. Marina Dobrovolskaia presented results of studies on the correlation between *in vitro* and *in vivo* immunotoxicology in support of preclinical development of nanotechnology-formulated complex drugs. They demonstrated the following: *good correlations* in hemolysis, complement activation, pyrogenicity, cytokine induction, and mononuclear phagocyte system (MPS) uptakes; *fair correlations* between *in vitro* and *in vivo* immunotoxicology tests for thrombogenicity and myelosuppression; and *poor correlations* for immunosuppression and delayed typed hypersensitivity. Dr. Clarice Hutchens summarized the structure and operations of the Nanomedicines Alliance. This organization focuses on scientific advancement, advocacy with legislators and regulatory authorities, public appreciation of nanotechnology-based medicines, and publications. Dr. Jan Simak focused on studies addressing effects of nanomaterials on thrombosis and hemostasis. He summarized currently available characterization methods that are used for hemolysis, platelet aggregation, and plasma coagulation. He recommended research on the evaluation of size distribution, particle aggregation, surface charge, and anti-coagulation effects, since they have potential effects on thrombosis and hemostasis.

7.5.3. Panel Discussion

7.5.3.1. Documentary standards of interest

One challenge that requires research, improved understanding, and eventually standards is relates to the mechanisms of action for targeted nanomaterials:

- Recent studies indicate that macrophages play an active role in (targeted) delivery of nanotherapeutics acting as intermittent 'depots' that capture and then gradually release nanomaterials and or drug carried by nanomaterials. Is the anti-cancer action mediated by targeted delivery in that case?

- In many tumors, the targeted nanomaterial construct is trapped inside stroma barriers and releases drug in stroma. Again, does the anti-tumor action benefit from targeting in that case?

Targeting adds another dimension of complexity to the evaluation of nanomaterial-based drugs:

- Characterization methodologies need to evaluate biological activity of nanomaterial-containing drugs
- Can we effectively characterize targeting ligand surface density and its distribution? Do we know how many of those targeting ligands are active?
- Further need for efforts to correlate results from *in vivo* and *in vitro* studies.

7.5.3.2. Challenges and considerations

Challenges and comments that were highlighted from the discussions include:

- Drug products containing nanomaterials should be well-characterized and designed to be specific such that non-target exposure is minimized and liver nanomaterial accumulation reduced.
- Highly sensitive standard methods need to be developed to assess immunological activities of nanomaterials.
- Using macrophages as 'depots' to capture and gradually release nanomaterials may be a viable delivery strategy. This will allow macrophages to help deliver therapeutic agents into target organs by passing the stroma barrier surrounding tumor cells, and distribute the drugs over longer periods of time.
- Nanomaterial properties can promote accumulation in certain locations without using active targeting moieties.
- Need for optimization of experimental conditions of the hemolysis, platelet aggregation, and plasma coagulation tests to mimic real human exposures (*in vivo*)

7.5.3.3. Prioritized regulatory science research and standards needs

The following were identified as priorities for RMs and standard method development:

- Complement activation: good *in vitro-in vivo* correlation, predictive of complement activation-related pseudoallergy (CARPA), established with FDA-approved drugs causing CARPA in clinical settings
- Cytokines secretion: good *in vitro-in vivo* correlations, predictive of cytokine storm and pyrogenicity (TNFa, IL-6, and IL-1b), validated in clinic with TGN1412 antibody products
- Blood partitioning: predictive measures of quick drug release in blood (and indirectly of efficacy) following systemic administration
- Uptake by monocytes in whole blood: predictive of stealthiness and biodistribution to MPS (indirectly of efficacy and off-target toxicity)

7.5.3.4. Overall conclusions of discussions

The key conclusions of the discussion held at the end of the session were:

- Proper characterization of chemical characteristics of drug products containing nanomaterials needs to be further developed.
- Design work flow of drug products containing nanomaterials needs to include targeting ligand and targeted receptors.
- Characterization of endotoxins and other immunotoxicity effects is required.
- Further standardization of methods for quality control of products is needed.

7.6. Nanomaterials in Personal Care Products

Co-chairs: Dr. Nakissa Sadrieh and Dr. Nigel Walker

7.6.1. Introduction

Nanomaterials are used in cosmetic products for various effects. Probably the most common claim for the use of nanomaterials in cosmetics is to enhance the delivery of cosmetic ingredients into the skin. Their small size is thought to enable some nanomaterials to be absorbed more readily into skin, while the unique surface properties afforded by the nanoscale have fueled claims of novel effects; however, unlike for drugs, assessing the safety of cosmetic products is the responsibility of the manufacturer. This has raised potential safety concerns, given the lack of regulatory requirements for premarket testing, as well the lack of a scientific consensus on "what" and "how" to evaluate the safety and quality of nanoscale cosmetic ingredients and cosmetic products that contain such nanoscale materials. Some of these concerns could be addressed if there were appropriate and widely accepted and available methods for measuring the critical attributes of nanomaterials, as well as their safety profile, in bulk material, as well as in formulated products. Critical attributes can include parameters such as the relevant physico-chemical properties of nanomaterials used, their stability in formulated products, their potential for skin absorption, their potential for other possible unintended routes of exposure, and whether their properties, including bioavailability and toxicity, can change when they are formulated in cosmetic products. There has been considerable effort internationally to harmonize approaches, but definitional issues and safety concerns related to the use of nanomaterials in cosmetic products remain to be addressed. Some guidance documents have been published to date, addressing the need to consider multiple factors when formulating cosmetics with nanomaterials; however, the value of the existing documents is limited with respect to how to specifically carry out acceptable assessment of the safety and quality of cosmetic products formulated with nanomaterials.

7.6.2 Speakers' Presentations

Dr. Nakissa Sadrieh presented a summary of the current US regulations of cosmetics and how that applies to nanomaterials. A key point raised was that, in contrast to drug development, FDA authority for cosmetics is post-market and hence there is no authority for premarket

approval for safety or efficacy. Consequently, the producer bears the responsibility for safety of cosmetics containing nanomaterials and the burden of proof would be for FDA to show harm. Dr. Birgit Sokull-Klüttgen presented a summary of the current EU perspective for cosmetics. One of the challenges noted was that this field is highly dynamic and that approximately every five years, 25% of products are "reformulated". Also, in the EU, there is a six-month prior marketing notification for cosmetic products which contain nanomaterials that can trigger an assessment of safety of these ingredients via a Scientific Committee. In addition, and in contrast to the US, there is a labelling requirement if a product contains "nanomaterial" ingredients. This is based on a legally binding definition of "nanomaterial" in the Cosmetic Products Regulation (from 2009). This definition is different from the EU definition of nanomaterial (from 2011) based on a "50% threshold in the particle number-based particle size distribution in the 1 nm to 100 nm range"; however, alignment of these two definitions is foreseen. A great challenge for the EU regarding regulation of nanomaterials is the implementation of the definitions and the ingredient labelling requirements. Dr. David Andrews provided an overview of the Skin-Deep Database on sunscreens developed by the Environmental Working Group as a tool for consumers. It was noted that the data used to create this database is based on product labels and has not been independently verified by experimental analyses. The database covers 62,000 products and includes the ingredients in these products, but not the ingredient concentration or physical form. This is a highly visible database: in the past 10 years this database has received 600 million page views. With respect to nanomaterials, products are included that contain titanium dioxide, zinc oxide iron oxide, boron nitride, and silver; no current products specifically self-identify as being "nano". Sunscreens have been a focus over the past 10 years with mineral actives (titanium dioxide and zinc oxide). It was noted that for cosmetic appearance, the titanium dioxide would need to be in the 10 nm to 30 nm range; zinc oxide can be larger (approximately 150 nm). Dr. Monita Sharma outlined some of the challenges with assessing the safety of nanomaterials and the application of *in vitro* technologies to these assessments. It was noted that there are new *in vitro* predictive approaches for skin sensitization of chemicals that perform better than animal-based tests, and are based on AOPs. These approaches have not yet been tested for applicability for use with nanomaterials. It was noted that there is a large quantity of emerging *in vitro* data on nanomaterials; however, there is a need to allow integration of data from multiple studies by systematic reviews of this literature. Dr. Shou-Chieh Huang discussed exposure assessment for the use of nanoparticles in sunscreens, particularly attempts at characterizing nanoparticles in formulated products and sunscreen sprays. This talk noted also the need for guidance on electron microscopy of nanomaterials, and on how to effectively measure the relative concentration of nanomaterials in a product. Dr. Sri Nadadur provided an overview of research funded by NIEHS on a variety of nanomaterials found in personal care products, with a focus on emerging research findings on nano-silver. He also discussed the benefit of guidance documents, citing the example of development and use of a "standardized protocol" for cytotoxicity assessments of nanomaterials *in vitro* by a consortium of academic scientists.

7.6.3. Panel Discussions

7.6.3.1. Documentary standards of interest

Standards that are needed were discussed and placed in a framework reflecting the timeframe of the need and types of need (standards or research).

7.6.3.1.2 Short-term needs

RMs

- Size distribution by number

Guidance documents

- Survey of methods/assays available and applicability to nanomaterials
- How to perform representative "sampling" of products in complex media
- Impact of nano-labelling for consumers and regulators
- Detection, identification, characterization and quantitation of nanomaterials in complex matrices

Research "needs"

- Methods to characterize ingredients
- Characterization by *in vitro* tests
- Data to aid groupings for "read across" of safety
- *In utero*/perinatal exposures
- Guidance on what specific materials to focus on

7.6.3.1.2 Medium-term needs

RMs

- None identified

Guidance documents

- Applicability of guidance on "read across" for materials to "read across" for nanomaterials
- Dermal penetration of nanomaterials
- Detection and quantitation to identify nanomaterials in complex media
- Nanomaterial number concentration in a sample

Research "needs"

- Environmental impacts of nanomaterials in personal care products
- AOPs directed "Testing batteries"
- Studies on health effects in populations

7.6.3.1.3 Long-term needs

RMs

- None identified

Guidance documents

- AOPs and linkages to "harm" for regulatory decisions/public decisions

Research "needs"

- None identified

7.6.3.2. Challenges and considerations

The products that are covered in personal care are highly diverse. Some of the overarching themes from the talks and discussions were:

- Differences in the regulatory framework between the US and EU
- Challenges for measurement of nanomaterials as isolated ingredients versus in products
- Importance of characterization as it pertains to product labelling and use of the information in the labels
- Applicability of alternate AOP-driven *in vitro* assays for assessing nanomaterial hazard and adaptation of such assays for use with nanomaterials

7.6.3.3. Prioritized regulatory science research and standards needs

The following specific research and standards needs were identified:

- Detection, quantification and characterization of nanomaterials in complex matrices
- Guidance on electron microscopy analysis of nanomaterials and measurement of "percent nano"
- RMs and standard test methods for detection/quantitation below "10nm"
- Review of existing *in vitro* assays for applicability for nanomaterials
- Research and guidance on applicability of new *in vitro* "skin sensitization" assays to nanomaterials
- Research and guidance on dermal penetration of nanomaterials
- Applicability of *in vitro* assays for nanomaterials in specific assays for AOPs (*e.g.,* pulmonary fibrosis, skin sensitization)

8.0 References

1. Bangham AD, Horne RW. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. J. Mol. Biol. 1964, 8:660-668.
2. Barenholz, Y. Doxil®- the first FDA-approved nano-drug: lessons learned, J. Control. Release 2012, 160: 117–134.
3. FDA (US):
   a. Draft Guidance for Industry: Liposome Drug Products Chemistry, Manufacturing and Controls; Human Pharmacokinetics and Bioavailability; and Labelling Documentation (2002, 2015),
   http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070570.pdf ;
   b. Draft Guidance on Doxorubicin Hydrochloride,
   http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM199635.pdf ;
   c. Draft Guidance on Amphotericin B,
   http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM384094.pdf ;
   d. Draft Guidance on Verteporfin,
   http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM384173.pdf.
4. MHLW (Japan);
   a. Guideline for the Development of Liposome Drug Products, 2016,
   http://www.nihs.go.jp/drug/section4/160328_MHLW_liposome_guideline.pdf
5. EMA (EU);
   Reflection paper on the data requirements for intravenous liposomal products developed in reference to an innovator liposomal product,
   http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/03/WC500140351.pdf
6. Chaudhry, Q. and Castle, L. (2011) Food applications of nanotechnologies - An overview of opportunities and challenges for developing countries, Trends in Food Science and Technology 22 (2011): 595-603.

9.0 Tables

Table 1. Examples of ISO **and** ASTM standards, technical reports, and guides that can facilitate safety assessments of nano-enabled medical devices (section 7.2.1) products

	Standards, Technical Reports, and Specifications	ISO Technical Committee 194 on Medical Devices Biological and clinical evaluation of medical devices	Application	Relevance to Regulators	Comments
1	TR10993-3	Biological evaluation of medical devices — Part 3: Tests for genotoxicity, carcinogenecity, and reproductive toxicity:	Biological testing	Yes	Needs for considerations and raising awareness for evaluation of genotoxicity of nanomaterials
2	TR10993-22	Biological evaluation of medical devices — Part 22: Guidance on nanomaterials	General considerations	Yes	General considerations and awareness raising for evaluation of genotoxicity of materials that may be extensible to nanomaterials associated with medical devices
		ISO Technical Committee 229, Nanotechnologies	**Application**	**Relevance to Regulators**	**Comments**
3	TR 16196	Guidance on factors to consider regarding sample preparation and dosing for engineered and manufactured nanomaterials	Biological testing	Yes	Important considerations for sample preparation and dosing of nanomaterials for in vivo and in vitro models
4	TR 16197	Compilation and description of toxicological screening methods for manufactured nanomaterials	Biological testing	Yes	Description of assays for safety evaluation of NMs
5	DTS 18827 TS 19006 DIS 19007	Standards, specifications and draft standards on biological testing, e.g., cytotoxicity, ROS	Biological testing	Yes	Toxicity testing of specific endpoints indicated (ROS production, cytotoxicity)

		production, genotoxicity			
6	TR 13014	Guidance on physico-chemical characterization of engineered nanoscale materials for toxicological assessment	Risk Assessment	Yes	Toxicity testing
7	TR 13121	Nanomaterial risk evaluation	Risk Assessment	Yes	Risk assessment
8	TS 11931	Nanoscale calcium carbonate in powder form -- Characteristics and measurement	Particle Characterization	Yes	Potential applicability to medical devices containing calcium carbonate as NM
9	ITS 11937	Nanoscale titanium dioxide in powder form — Characteristics and measurement	Particle Characterization	Yes	Potential applicability to medical devices containing TiO_2
10	TS 14101	Surface characterization of gold nanoparticles for nanomaterial specific toxicity screening: using Fourier-transform - infrared method	Particle Characterization	Yes	Potential applicability to medical devices containing gold nanoparticles
11	29701	Endotoxin test on nanomaterial samples for in vitro systems — Limulus amebocyte lysate (LAL) test	In vitro method to measure endotoxins	Yes	In vitro endotoxin test
	ASTM International Standards	**ASTM Technical Committee E56, Nanotechnology**	**Application**	**Relevance to Regulatorsregulators**	**Comments**
12	ASTM E2524-08	Standard Test Method for Analysis of Hemolytic Properties of Nanoparticles	Blood contact properties	Yes	Human health related
13	E2525-08	Standard Test Method for Evaluation of the Effect of Nanoparticulate Materials on the Formation of Mouse Granulocyte-Macrophage	In vitro method to test for inhibition of bone marrow stem	Yes	In vitro test for stem cell inhibition

		Colonies	cells		
14	ASTM E2526-08	Standard Test Method for Evaluation of Cytotoxicity of Nanoparticulate Materials in Porcine Kidney Cells and Human Hepatocarcinoma Cells	In vitro method to measure cytotoxicity	Yes	In vitro cytoxicity test
15	E2535-07	Standard Guide for Handling Unbound Engineered Nanoscale Particles in Occupational Settings	Guide to safe handling in occupational setting	Yes	Occupational hazards
16	E3025-16	Standard Guide for Tiered Approach to Detection and Characterization of Silver Nanomaterials in Textiles	Guide to testing of nano-enabled textiles	Yes	Wound dressings
17	E2490-09	Standard Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Photon Correlation Spectroscopy (PCS)	Particle size measurements	Yes	Recognized by FDA
18	ASTM E2864-13	Standard Test Method for Measurement of Airborne Metal and Metal Oxide Nanoparticle Surface Area Concentration in Inhalation Exposure Chambers using Krypton Gas Adsorption	Test method for airborne nanomaterials	Yes	Assesses exposure in occupational and other environments

Note: TS=Technical Specification; DTS=Draft Technical Specification; TR=Technical Report; DIS=Draft International Standard

Appendix A:

Perspective on the Regulatory Approval Process in the EU, and the Impact of the GSRS Meetings by Fergal Donnelly, M.D., EC. (European Commission Directorate of Health, EU)

Regulatory approval systems for the marketing of healthcare products must protect the public and at the same time, they must ensure appropriate and timely access to the latest products and interventions. Yet despite significant increases in investment into in research and innovation in healthcare, the output at the end of the pipeline remains stubbornly low in getting new innovative products to the patient bedside. An escalation in costs, additional regulatory hurdles and the sector being severely impacted by austerity measures resulting from the economic crisis in Europe since 2010 has made the realisation of this objective seem daunting.

At the same time, healthcare interventions have become increasingly complex as a reflection of input from different scientific fields and novel technologies in the healthcare sector. Their rapid evolution and convergence that often result in a single functioning entity require the constant addition and adaptation of involved stakeholders, including drug regulators. Data and technical requirements required are complex and increasingly interlinked with one another and are accompanied by high public expectations. There are also inherent uncertainties that are associated with the natural evolution of science.

There is a sea change in the demographic profile in Europe – as in all other parts of the world – that by the year 2050 the number of people – not just in the EU but everywhere – aged 65 or more will have grown by at least 70% and for those in the 80+ age group by at least 170%. An ageing population is more prone to illness, and therefore, in order to keep people healthy and active, and at the same time manage healthcare costs, is a growingly important societal challenge.

In order to ensure public protection while at the same time ensure that the best possible healthcare interventions remain affordable to patients, a solid scientific and technical foundation – or regulatory science - is required for the regulation and marketing of healthcare products. This aims to harness science in evolution, resulting in better policies for marketing authorisations and in as transparent and open a way as possible.

The research and industrial markets reflect the somewhat fragmented nature of the regulatory processes that govern the marketing of medical devices and other related healthcare products. Like pharmaceutical products, Advanced Therapies are regulated centrally by the European Commission in association with the European Medicines Agency and in particular, the Committee for Advanced Therapies (CAT).

This is the European Medicines Agency's (EMA) committee that reviews the quality, safety and efficacy of these products as well as follow scientific developments in the field. It garners the best available expertise in Europe and proposes a draft opinion on each ATMP application submitted to EMA. Based on this, the Committee for Medicinal Products for Human Use (CHMP) then issues its final opinion on the marketing authorisation of the product concerned. This latter opinion forms the basis of each Marketing Authorisation that is issued by the European Commission.

Scientific Advice is available from the European Medicines Agency, so as to anticipate potential major objections regarding their design and content that may occur during the review of the marketing authorisation application.

A major element of scientific advice is a risk-benefit assessment and an economic evaluation. This forms the basis of Health Technology Assessment, which is a systematic, multi-disciplinary and economic evaluation of the properties and effects of a healthcare product or service, addressing its direct and indirect, intended or unintended effects. One of its major uses is to determine reimbursement policy on a case by case basis and coverage decisions by individual Member States.

A useful but under-used regulatory procedure that has also resulted from the scientific advice procedure is the acknowledgement of the acceptability of research data as part of a marketing authorisation application. This data certification does not mean acceptance and approval of the data per se, but rather an acknowledgement that it has been generated according to scientifically sound methodologies based on standard acceptable clinical practice.

Medical Devices on the other hand are marketed in the EU subject to the awarding of the CE mark from individual Member States' Notified Bodies. These entities are officially accredited to determine whether products conform to EU Medical Devices Directives, for which revisions have been proposed and if so, that they can then be marketed in the EU. These authorisations are also subject to Health Technology Assessment as above.

Regulatory Science can facilitate a better- informed decision-taking process regarding the marketing of healthcare products. A lifecycle management approach to products is needed for

a maximal protection of the public that takes account of the evolution of science along the following lines:

- Harmonisation of various regulations and other legislative provisions that govern pharmaceutical products, Advanced Therapy Medicinal Products and Medical Devices so as to facilitate new product innovations. This should as far as possible reflect the work of the ICH (International Conference on Harmonisation),
- Foster constructive dialogue between key stakeholders in the research and innovation process. These comprise *inter alia* both centralised and individual Member State regulatory bodies, notified bodies, marketing authorisation applicants, Health Technology Assessment bodies, pricing and reimbursement entities and health insurance organisations,
- Develop and pilot new science-based methods, models and industrial standards for a better determination of efficacy and earlier determination of risk. These can be based on data mining exercises across the research spectrum on a given healthcare product and between different regions of the world,
- Extend data certification procedures to all parts of the research and innovation pipeline and solidify links with marketing authorisation procedures as value-added milestones in this process,
- Invite patient representative groups to contribute to the definition of efficacy and safety criteria that are needed for marketing authorisations.

Prioritized Regulatory Science Research and Standards Needs

A scientific basis for the better regulation of healthcare products is required for a number of reasons.

Firstly, the shift in the role of regulators from being solely gatekeepers towards maintaining the balance between public safety and facilitating the needs of innovation is part of the transition from the present form of a binary yes/no approach towards the concept of an evolving lifecycle management. This requires a more efficient interaction between regulators, academia and industry throughout the lifetime of products, both before and after marketing.

Secondly, regulatory science is needed so as to cross the so called “valley of death” between pure laboratory-based research on the one hand, and the ideal method of clinical testing on the other. This gap can arise for many reasons (lack of definition of the final target patient population, incomplete preliminary technical data). Translational Medicine, which is a better application of the knowledge gained from the laboratory in the clinical testing sphere and which results from better interaction between researchers above, is an integral part of closing this gap.

Part of this means having better education and training so as to produce individuals who understand both medical science and the related commercial or non-scientific aspects that are part of the research and innovation process. Examples comprise healthcare economics, ethics, management, business administration, and law. Better data-based modelling systems that harness information from these domains can thereby be devised leading to a better understanding of the overall challenges and thereby encourage stronger innovation.