For Official Use **ENV/CHEM/NANO/RD(2015)4**

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development **30-Oct-2015**

English - Or. English

ENVIRONMENT DIRECTORATE
CHEMICALS COMMITTEE

ENV/CHEM/NANO/RD(2015)4
For Official Use

Working Party on Manufactured Nanomaterials

WPMN INFORMATION SHARING SEMINAR ON IN VIVO INHALATION TOXICITY SCREENING METHODS FOR MANUFACTURED NANOMATERIAL

DRAFT SUMMARY RECORD

21 September 2015, Washington DC, USA

Mrs. Mar Gonzalez, Tel: 01 45 24 76 96, Email: mar.gonzalez@oecd.org

JT03385450

Complete document available on OLIS in its original format

English - Or. English

"WPMN INFORMATION SHARING SEMINAR ON *IN VIVO* INHALATION TOXICITY SCREENING METHODS FOR MANUFACTURED NANOMATERIAL"

Washington DC, USA on 21 Sept 2015

Draft Summary Record

TABLE OF CONTENTS

INTRODUCTION

1. The WPMN information sharing seminar on in vivo inhalation toxicity screening methods for manufactured nanomaterial was held on 21 September 2015 at US EPA headquarters in Washington, D.C. United States. The meeting was chaired by Japan, who opened the meeting and welcomed the participants. Participants were from France, Germany, Japan, Korea, the Netherlands, United Kingdom, United States, European Chemicals Agency (ECHA), industry (BIAC), environment and animal welfare NGOs and the Secretariat to the WPMN. The list of participants is available in Annex II.

RECENT DEVELOPMENT AND APPLICATION OF SHORT TERM INHALATION STUDY

"Short-Term Inhalation Study (STIS) as a range finder and screening tool in a tiered grouping strategy" *Lan Ma-Hock, Head of Inhalation Toxicology, BASF SE, Germany*

2. Short-term inhalation study employs a physiological way of administration. The study design presented covered the following key elements:

1. Inflammation potency at the respiratory tract;
2. Potential reversibility or progression of the effects; and
3. Deposition and clearance kinetics.
4.

3. Based on a few case studies, the application of short-term inhalation study as screening tool and range finder was presented. In the general discussion, the rationale for choosing recovery period of 3 weeks was questioned, mantioning the fact that the physiological clearance half-time is between 60 and 70 days. The answer was: it is a pragmatic approach to collect information on effect reversibility and clearance rate. As the method is introduced as a screening tool for grouping approach or as a range finder for sub-chronic studies, it is justified not to have the complete clearance kinetic, nor is a full reversibility of the effects required.

"Use of short-term inhalation study to obtain initial hazard data and to prepare subacute and subchronic inhalation studies"

Il Je Yu, Institute of Nanoproduct Safety Research, Hoseo University, Korea

4. A further thought to use the short-term inhalation study was introduced by Korea. He pointed out that an acute inhalation study according to OECD TG 403 did not provide valuable hazard information of nanomaterials. Instead of the acute inhalation study, a short-term inhalation study may be performed. This

type of study provides initial hazard information on nanomaterials and kinetics. Based on these data, concentration selection and study design may be established for the subsequent repeated dose inhalation studies. Interestingly, in the case studies presented, bronchoalveolar lavage was not as sensitive as histopathology. In the general discussion, the importance of histopathology was emphasized.

"Comparing short-term pulmonary bioassay studies with longer-term inhalation studies: What are the Advantages and Disadvantages?"

David Warheit, Chemours, USA

5. The speaker compared the screening studies with the inhalation studies. The first talk compared short-term pulmonary toxicity assay with long-term (90-day) inhalation studies. The fundamental tenets of pulmonary toxicology were presented:

1. Robust characterization of the test material;
2. Implementation of a dose/response regimen;
3. Time course characteristics; and
4. Inclusion of benchmark (reference) test materials (positive or negative controls).

6. Appropriately designed sub-chronic 90-day inhalation study covers most of these aspects. It is so labor and cost intensive that a benchmark control group or an additional examination time point often cannot be included. In comparison to the 90-day inhalation study, instillation study is more flexible with regards of simultaneous testing of several materials including benchmark controls. Testing of several post-exposure time points are also very common. Still, sub-chronic 90-day study is the golden standard for setting occupational exposure levels for humans and is the more physiological way of administration. This talk emphasized a long post-exposure observation period of 90-days. The duration of the post-exposure period was the main issue of the discussion. It was also mentioned that use and the interpretation of pulmonary screening bioassays in regard to risk assessment i.e. at the workplace can be supported by "bridging" the results to previous performed subchronic inhalation studies.

RECENT DEVELOPMENT AND APPLICATION OF INTRATRACHEAL ADMINISTRATION STUDY

"Pulmonary inflammatory responses following intratracheal administration and inhalation exposure of nanoparticles"

Yasuo Morimoto, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health (UOEH), Japan

7. Comparing instillation studies with 4 week inhalation studies showed that the instillation studies were able to differentiate the pulmonary inflammation potency of NiO, CeO_2 and TiO_2. The following questions were discussed:

1. Did you compare the results with in vitro studies?;

2. Dispersion of the materials;
3. Distribution within the lung after inhalation and instillation; and
4. Clearance rate after inhalation and instillation.

8. The speaker answered that his group did not perform in vitro study and did not compare the in vivo data with in vitro data. For inhalation and instillation studies, the test materials were dispersed in medium. The suspension was sprayed to generate aerosol in inhalation studies. The particle morphology in the media and in the lung differed from each other. The instillation achieved distribution in all lung lobes although more in the center focal region. The distribution after inhalation was more homogenous among the peripheral region. The clearance was comparable with slower clearance (e.g. CeO_2) after instillation than inhalation. Overall, instillation was considered being appropriate to be used as a screening tool to rank pulmonary toxicity of nanomaterials.

"Lung lesions induced by intratracheal administration of nanomaterials in rats: Their differences in treatment frequencies and comparison with inhalation exposure"

Shoji Fukushima, Director, Japan Bioassay Research Center, Japan Industrial Safety & Health Association, Japan

9. This talk focused on the administration regime of the instillation. Effects after single application was compared with those of 2, 3 or 4 instillations achieving the same final dose. For TiO_2 there were no differences between the treatment regimes. Based on bronchoalveolar lavage data, the toxicity of NiO increased with the frequency. However, histopathology did not reveal any differences in severity. Thus, single intratracheal administration was considered appropriate for hazard identification. Instillation of MWCNT was indicative for outcome after inhalation exposure. The general discussion was focused on data of MWCNT:

1. Is there difference in size of CNT between IH and IT studies?
2. Was fibrosis found in both studies?
3. Was the suspension of CNT for IT prepared by collecting aerosol used for IH study?

10. The answers were: it seemed that the sizes in inhalation and instillation studies were comparable. Fibrosis was found in both inhalation and instillation studies with MWCNT. In instillation study MWCNT was first dispersed in media prior to instillation. Both administration methods employed essentially different sample preparation before application. It was pointed out that distribution after instillation is uneven. It depends sample preparation and administration technique and encounters this shortness. Data evaluation and interpretation should consider the unevenness of the material deposition. It is recommended to use area under the curve as a metric of dose.

"Standardization of protocol"

Yutaka Oshima, Chemical Evaluation and Research Institute (CERI), Japan

11. The next talk worked out technical details of intratracheal instillation including anesthesia, administration device, procedure, instillation volume, concentration of the formulation. Addressing the above-mentioned technical issues, the most appropriate protocol for nanomaterials was suggested. The question was again, whether the distribution within the lung was homogeneous. As shown before, the distribution within the lung after instillation was not homogeneous as after inhalation. However, since the distribution was found reproducible to some extent, the results can be reasonably interpreted.

DISCUSSION ON HARMONIZATION AND STANDARDIZATION OF THE METHODS

12. The last talk is the connection to the discussion with regard to harmonization and standardization of the methods. At the very beginning phase, it was clarified that "harmonization and standardization" did not mean development of a regulatory-accepted new test guideline. The introduced short-term bioassays were intended to be used as screening and range finding studies. There was general agreement among the attendees that even for a screening tool, certain standardization is essential, that the data collected by academia and industry were reproducible and interpretable. The representative of regulatory bodies (BfR and ECHA) would welcome harmonized and standardized screening tools collecting more hazard information on different nanomaterials in order to apply grouping approach and enable risk assessment based on a solid data base. There were concerns of feasibility of standardization of intratracheal instillation on the current stage. Up to now, very few technical details were examined in detail. It was pointed out that more effort is necessary before standardization process can be initiated. Moreover, reviewing existing data, the variation of brochoalveolar lavage procedure were significant. However, most of the reported methods did lead to reasonable results, with exception of very few institutes. Is standardization still necessary?

SUGGESTIONS FOR GUIDANCE AND FRAMEWORK OF DISCUSSED SCREENING METHODS

13. It was suggested to achieve a standardized protocol within ISO. The majority of the attendees consider ISO as one possible option to standardize technical details. However, OECD was considered a more appropriate platform for application and the interpretation of the screening data. A guidance for use of screening tools i.e. in an integrated testing strategy and on interpretation of screening data (i.e. for hazard ranking, but also to predict possible long-term toxicity) may be helpful for risk assessors and regulators.

TECHNICAL DISCUSSION

14. In a more technical discussion, the exposure techniques of inhalation versus intratracheal instillation were compared. It was stated, inhalation and instillation have their legitimacy as screening tools. Inhalation reveals port of entry effects throughout the respiratory tract while instillation bypasses the upper respiratory tract and cause bolus effects. Based on the determined no effect concentration in the atmosphere, inhalation study can be used for regulatory purposes. However, inhalation study required elaborate equipment and thus cannot be performed easily. The available data indicated that despite of bolus effect after instillation, it reveals reliably the quality of the changes caused by the test substance, providing appropriate dose is used. Histopathological evaluation is essential.

ANNEX I. AGENDA

21st September 2015

8:30-8:35	**Opening remarks** *Masashi Gamo*, AIST, Japan
8:35-9:40 (65 min)	**Recent development and application of Short Term Inhalation Study (STIS)** • **Short-Term Inhalation Study (STIS) as a range finder and screening tool in a tiered grouping strategy** *Lan Ma-Hock*, Head of Inhalation Toxicology, BASF SE, Germany • **Use of short-term inhalation study to obtain initial hazard data and to prepare subacute and subchronic inhalation studies** *Il Je Yu*, Institute of Nanoproduct Safety Research, Hoseo University, Korea • **Comparing short-term pulmonary bioassay studies with longer-term inhalation studies: What are the Advantages and Disadvantages?** *David Warheit*, Chemours, USA
9:40-10:10	**Coffee Break**
10:10-11:15 (65 min)	**Recent development and application of Intratracheal Administration Study (ITAS)** • **Pulmonary inflammatory responses following intratracheal administration and inhalation exposure of nanoparticles** *Yasuo Morimoto*, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health (UOEH), Japan • **Lung lesions induced by intratracheal administration of nanomaterials in rats: Their differences in treatment frequencies and comparison with inhalation exposure** *Shoji Fukushima*,

	Director, Japan Bioassay Research Center, Japan Industrial Safety & Health Association, Japan • **Standardization of protocol** *Yutaka Oshima*, Chemical Evaluation and Research Institute (CERI), Japan
11:15-11:55 (40 min)	**Discussion** **Co-chairs**: *Masashi Gamo*, AIST, Japan and *Karin Wiench*, BIAC
	- What are the next steps for the better use of the screening methods? **- When can we make effective use of the methods as screening tools?**
11:55-12:00	**Closing remarks** *Karin Wiench*, BIAC

ANNEX II. PARTICIPANTS LIST

Participants list for WPMN information sharing seminar on in vivo inhalation toxicity screening methods for manufactured nanomaterials

21/9/2015 - 21/9/2015

France

Dr. Anne BRAUN
Head of Experimental Toxicology Unit
Experimental Toxicology Unit
National Institute for Industrial Environment and Risks (INERIS)

Germany/Allemagne

Mr. Otto CREUTZENBERG
Inhalation Toxicology & Chemical Risk Assessment
Fraunhofer Institute of Toxicology & Experimental Medicine (Fraunhofer ITEM)

Mr. Frank HERZBERG
Scientific Officer
Chemicals and Products Safety
Federal Institute for Risk Assessment (BfR)

Japan/Japon

Mr. Takuya IGARASHI
Senior Officer for Collaboration & Senior Researcher
Research Institute of Science for Safety and Sustainability (RISS)
National Institute of Advanced Industrial Science and Technology (AIST)

Dr. Masashi GAMO

Research Group Leader
Research Institute of Science for Safety and Sustainability (RISS)
National Institute of Advanced Industrial Science and Technology (AIST)

Dr. Kazuhiro YAMAMOTO

Research Group Leader
National Metrology Institute of Japan (NMIJ)
National Institute of Advanced Industrial Science and Technology (AIST)

Dr. Yasuo MORIMOTO

Professor
Dept. of Occupational Pneumology
University of Occupational and Environmental Health

Dr. Hiroto IZUMI

Associate Professor
University of Occupational and Environmental Health, Japan

Dr. Shoji FUKUSHIMA

Director
Japan Bioassay Research Center
Japan Industrial Safety and Health Association

Dr. Hirokazu KANO

Chief of Oral and Other Tests
Division of Experimental Toxicology, Oral and Other Test Section
Japan Bioassay Research Center, Japan Industrial Safety and Health Association

Mr. Yutaka OSHIMA

Pathologist
Section 2, Hita Laboratory, Chemicals Evaluation and Research Institute, Japan

Dr. Jun KANNO	*Division Head* *Division of Cellular and Molecular Toxicology, Biological Safety Research Center* *National Institute of Health Sciences (NIHS)*

Korea/Corée

Professor Il Je YU	*Professor* *Institute of Nanoproduct Safety Research* *Hoseo University*

Netherlands/Pays-Bas

Dr. Flemming. R. CASSEE	*Centre for Environmental Health Reaserch* *National Institute for Public Health and Environment (RIVM)*

United Kingdom/Royaume-Uni

Dr. Rachel SMITH	*Toxicology* *PHE*

United States/États-Unis

Dr. Iris CAMACHO	*Office of Pollution Prevention and Toxics* *US Environmental Protection Agency*
Dr. Kevin DREHER	*National Health and Environmental Effects Research Laboratory* *US Environmental Protection Agency*
Ms. Wanda HALL	*Program Analyst* *Office of Pesticide Programs/Field and External Affairs Division* *US Environmental Protection Agency*

Dr. Mark HIGUCHI	*Chief, Inhalation Toxicology Facilities Branch* *National Health and Environmental Effects Research Laboratory* *U.S. Environmental Protection Agency (EPA)*
Ms. Laura NAZEF	*Office of Chemical Safety and Pollution Prevention* *U.S. Environmental Protection Agency*
Mr. John WHALAN	*Office of Research and Development* *U.S. Environmental Protection Agency*

EU/UE

Dr. Camelia CONSTANTIN	*Juniro Scientific Officer* *Evaluation* *European Chemicals Agency (ECHA)*

Business and Industry Advisory Committee (BIAC)/Comité consultatif économique et industriel (BIAC)

Dr. Rodney J. BOATMAN	*Boatman Toxicology Consulting LLC*
Dr. Shaun CLANCY	*Director - Product Regulatory Services*
Dr. Yoshihito DEGUCHI	*Sumitomo Chemical America Inc.*

Dr. Maria DONNER	*Senior Research Toxicologist* *DuPont - Haskell Global Centers for Health & Environmental Sciences*
Dr. Lan MA-HOCK	*Experimental Toxicology and Ecology* *BASF SE*
Dr. David WARHEIT	*Research Fellow* *DuPont Haskell Laboratory*
Mr. Jay WEST	*Senior Director, Chemical Products and Technology* *American Chemistry Council*
Dr. Hans Juergen WIEGAND	*Global Coordinator Product Stewardship* *Evonik Industries*
Dr. Karin WIENCH	*Director of Regulatory Toxicology II* *Product Safety, GUP/PC Z-470* *BASF SE*

Environmental NGO

Mr. Martin MOELLER	*Senior Researcher (Produkte & Stoffströme)* *Öko-Institut e.V., Institut für angewandte Ökologie (OEKO)*

International Council on Animal Protection in OECD Programmes

Dr. Monita SHARMA	*Nanotoxicology Specialist* *PETA International Science Consortium (PISC), Ltd.*

Other/Autre

Dr. Philip SAYRE	*Owner* *nanoRisk Analytics*

OECD/OCDE

Ms. Mar GONZALEZ	*Administrator, Nanosafety, Chemical Accidents, and Outreach* *ENV/EHS* *OECD*