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Green Ribbon Science Panel Subcommittee #1: Chemical Identification and Prioritization

Attachments for April 13, 2011 Green Ribbon Science Panel Subcommittee 1 Meeting

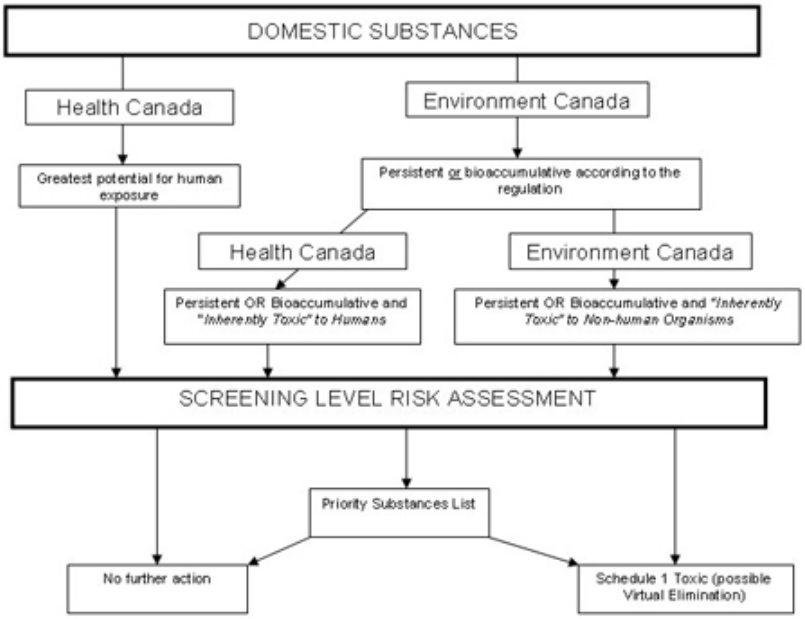
- GRSP Topic #1 Paper: Chemical Identification & Prioritization (3-28-2011)
- Canadian Prioritization Process
- ECHA Prioritisation Approach for SVHCs
- OECD Guidance for Nanomaterials Testing (2010)
- Setting Priorities for Drinking Water Contaminants (1999)

Prioritization of Existing Substances under the Canadian Environmental Protection Act.
OEHHA-COEH Workshop on Practical Decision Making Tools for Identifying Safer Alternatives
Sacramento, CA 10/2007
Presented by K. Hughes, Health Canada

Canadian Environmental Protection Act:

- Managing chemical substances is overseen by Canadian Ministers of Environmental & Health through CEPA.
- The Act provides the regulatory framework and process for risk assessment and risk management of chemicals through the **Domestic Substances List (DSL)**. The DSL includes:
    - Existing substances in commerce in Canada, excluding: contaminants, by-products and wastes
    - New substances: subject to data submission and review prior to introduction to commerce
- There is a 2-stage prioritization process for substances on the list: 1) initial categorization based on ecological and human health factors, and 2) more thorough screening assessment for high priority chemicals.

Figure 2: Categorization and Screening Process



(http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&n=E00B5BD8-1&offset=5&toc=show#522)

Categorization Criteria
CEPA 1999 required Ministers of the Environment and Health to categorize the 23,000 substances on the DSL according to specific criteria to identify substances that may present, to individuals in Canada:

- the **greatest potential for exposure or**
- are **persistent**(P) **or**
- **bioaccumulative**(B), in accordance with the regulations, **and**
- **inherently toxic** to humans or to non-human organisms, as determined by laboratory or other studies

Categorization process diagram: slide 8

Data Process:
Technical approaches and guidance for categories of types of chemicals, based on their class (associated workshops, advisory groups, and guidance material was generated for each):

- Organics (guidance 2003)
- Inorganics (guidance 2003)
- UVCBs (guidance 2005)
- Polymers (2005)

Data Sources: Experimental, US HPV, US OCED lists

Data preference diagram, slide 16

TOOLS FOR CATEGORIZATION:

Exposure Tools:

- Simple Exposure Tool: SimET: SimET is a relative ranking tool by which all substances on the Domestic Substances List have been "binned"
- Complex Exposure Tool: Provides plausible upper bound quantitative estimates of combined consumer (nearfield) & multimedia environmental (farfield) exposure by duration and age group, taking into account:
    - "Sentinel" product scenarios
    - Concentrations in environmental media of human exposure estimated based on extension of fugacity modelling

Graphic of complex exposure tool, slide 23

Hazard Tools:

- Simple Hazard Tool: SimHaz: Applied to entire DSL, a compilation of other sources' hazard determinations:
    - Defines high or low hazard from classifications/assessments of other agencies based on weight of evidence; Appropriate assessments selected based on comprehensiveness of review, peer review process, etc.
    - *High Hazard Lists/Endpoints*: Cancer (IARC, EU, HC, US EPA etc.), Genotoxicity (EU), Developmental Toxicity (EU), Reproductive Toxicity (EU)
    - *Low Hazard Lists:* PMRA 4a/US EPA 4a OECD Low Concern
    - Strengths: Efficient, Takes advantage of critical review of others, Consistency Assessments/classifications internationally
    - Limitations: Bias towards data-rich substances
- **ComHaz Tool**: Hierarchical approach to consideration of multiple endpoints relevant to characterization of hazard
    - Strengths: Health protective, Comprehensive, High confidence in "set asides", No bias towards data rich substances, Designed for high throughput, Takes advantage of critical reviews of others, Significant contribution of QSAR component to priority setting, External input, consultation, peer review
    - Limitations: Resource intensive
- **ComHaz Hierarchy slide 28**
- **ComHaz tool Endpoint specific criteria slide 30**

Time and Scope

- Categorization/Prioritization completed in September 2006
- Of the 23,000 substances on the DSL, 4300 identified as priorities, 4000 met the categorization criteria, 300 warrant further attention from a human health perspective

High Priority for Action:

- High priorities for action included substances that met each of the ecological categorization criteria (persistence (P), bioaccumulation (B) and inherent toxicity to aquatic organisms (iT), and believed to be in commerce in Canada and/or;
- That met the criteria for greatest or intermediate potential for exposure (GPE or IPE) and were identified as posing a high hazard to human health (i.e., classified by another agency on the basis of carcinogenicity, mutagenicity, developmental toxicity or reproductive toxicity as identified by SimHaz Tool)

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Prioritization of Existing Substances under the Canadian Environmental Protection Act

OEHHA-COEH Workshop on Practical Decision-Making Tools for Identifying Safer Alternatives

Sacramento, California
October 1-2, 2007

Presented by K. Hughes, Health Canada

Overview

- Background
  - *The Canadian Environmental Protection Act*
  - Existing Substances
  - The Domestic Substances List
- Categorization – A Priority Setting Exercise
  - Ecological
  - Human Health
- Canada's Chemicals Management Plan
  - The Challenge
- Screening Assessments

The *Canadian Environmental Protection Act*

- Covers a range of activities that can affect human health and the environment, and acts to address any pollution issues not covered by other federal laws.
- Managing chemical substances is a fundamental part of CEPA; shared by Ministers of Environment & Health
- Provides the regulatory framework and process for risk assessment and risk management of chemicals

Addressing Existing Substances under CEPA

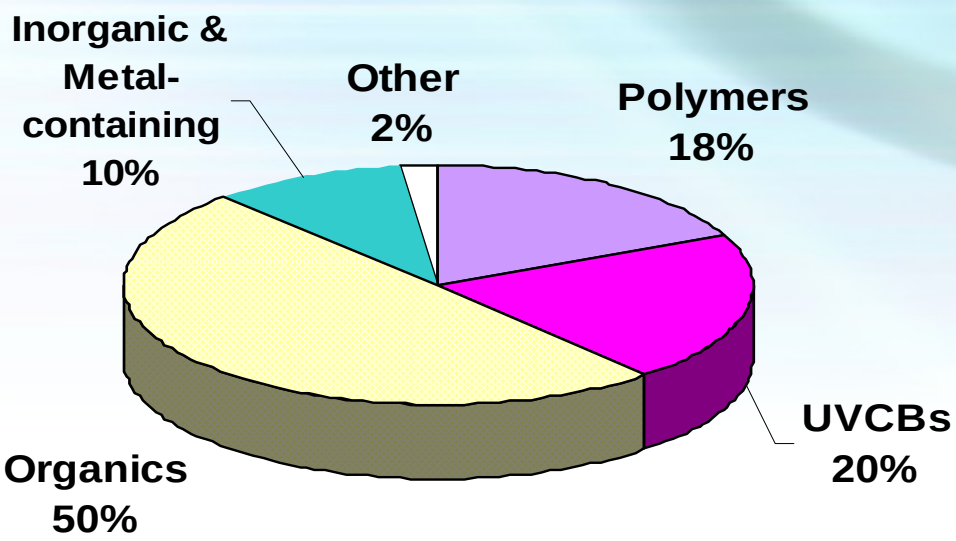
- CEPA 1988 (previous version)
  - Focused on pollution management
  - Priority Substance List assessments
    - In-depth, complex; 5 year legislated deadlines
    - PSL1 (n=44 substances, released in 1989)
    - PSL2 (n=25 substances, released in 1995)

- CEPA 1999 (revised in 1999)
  - Focus is on pollution prevention
  - Ministers' mandate expanded
  - Introduced Categorization of entire DSL (n = 23,000)
  - Screening assessments
  - Priority Substances List assessments

What is the Domestic Substances List (DSL)?

- A list of substances that are "in commerce" in Canada – "existing substances"
- It was created in 1991 with a list of substances which were between 1984-1986:
  - In Canadian commerce or used for commercial manufacturing in Canada, or;
  - Manufactured or imported in Canada at >100 kg/year
- DSL does not include: contaminants, by-products and wastes
- If a substance is not on the DSL, it is a "new substance", thus subject to data submission and review prior to introduction to Canadian commerce

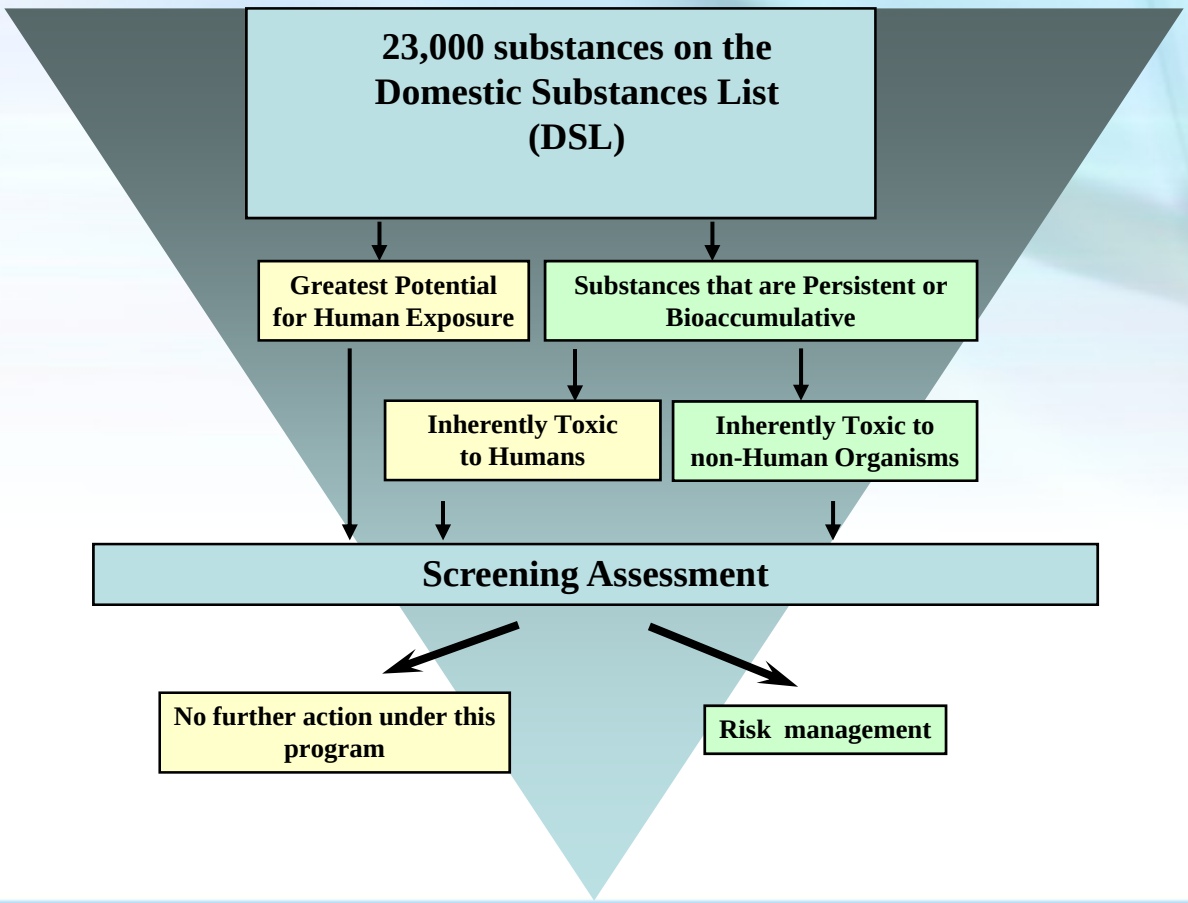
Types of Substances on the DSL



What was CEPA Categorization?

- CEPA 1999 required Ministers of the Environment and Health to categorize the 23,000 substances on the DSL according to specific criteria to identify substances that
  - May present, to individuals in Canada, the **greatest potential for exposure; or**
  - Are **persistent** (P) **or bioaccumulative** (B), in accordance with the regulations, **and inherently toxic** to humans or to non-human organisms, as determined by laboratory or other studies
- Categorization represented a priority setting exercise that involved the systematic identification of substances that should be subject to screening assessments and by extension management controls if applicable

The Categorization Process



What were the Categorization Challenges?

- No precedent, leading development of methodology
- Legislated deadline (7 years)
- Large number of substances had limited or no empirical data
- Varied types of substances on DSL
- Needed to develop an approach that was protective, transparent, scientifically credible

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Ecological Categorization Criteria for P, B, and non-human iT

Bioaccumulation

BAF $\geq$ 5000
or
BCF $\geq$ 5000
or
log Kow $\geq$ 5

iT –non-humans

Acute aquatic toxicity of $LC(EC)_{50} \leq$ 1mg/L, or a chronic aquatic toxicity of NOEC $\leq$ 0.1 mg/L

Persistence

A substance is considered persistent if its transformation half-life satisfies the criterion in any one environmental medium or if it is subject to long-range transport

Medium	Half-life
Air	$\geq$ 2 days (or LRT)
Water	$\geq$ 6 months
Sediment	$\geq$ 1 year
Soil	$\geq$ 6 months

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Process for Ecological Categorization

Define Technical Approach (Guidance Manual)

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Collection of Empirical Data and Generation of QSAR predictions*

**released publicly on CD*

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Scientific Evaluation of Data

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Release Preliminary Categorization Decisions*

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Process for Ecological Categorization (cont'd)

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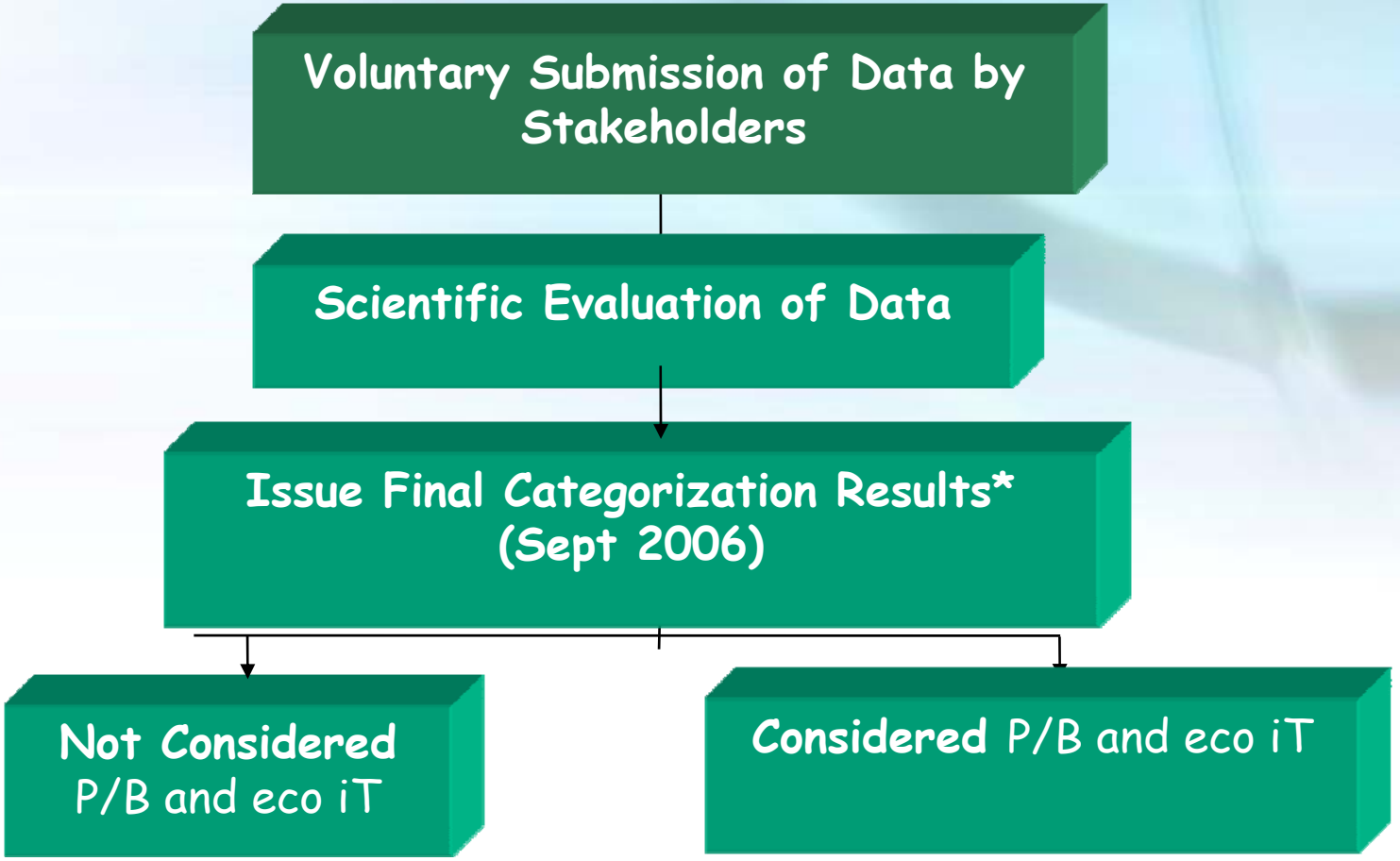

Key Areas of development for Technical Approaches and Strategic Guidance

- Organics
  - DSL Technical Advisory Working Group (1999-2001)
  - October 2002 Technical Workshop
  - Guidance Manual for the Ecological Categorization of Organic and Inorganic Substances on the DSL (2003)
- Inorganics
  - Inorganics Working Group (IWG) (2000-2001)
  - Findings and Recommendations from the IWG Report (2001)
  - Guidance Manual for the Ecological Categorization of Organic and Inorganic Substances on the DSL (2003)
- UVCBs
  - Golder Associates' Report on Developing an Approach for UVCBs (2003)
  - Boreal Associates' Report on Developing an Approach for UVCBs (2004)
  - Approach Document for Ecological Categorization of UVCBs (2005)
  - Category Approaches Documents (2005)
- Polymers
  - Approach Document for Ecological Categorization of Polymers (2005)
  - Category Approaches Documents (2005)
- Organometallics
  - Approach Document for Ecological Categorization of Organometallics (2005)

Information Sources

- Information Sources
  - Publicly available databases, journals, internet, international lists and data sources
  - Voluntary data submitted by Industry
  - Generated some phys-chem data and ecotoxicity data
  - Modelled data - QSARs (Quantitative Structure Activity Relationships)

Availability of Empirical Data

- Availability of Empirical Data
  - For more than 11,500 organic substances examined,
    - Experimental aquatic toxicity data was found for 1200 substances (80% accepted)
    - Experimental P data was found for 1500 substances (50% accepted)
    - Experimental B data was found for 440 substances (80% accepted)
  - 2100 substances on the DSL are also part of the US HPV program and 3140 are part of the OECD HPV program
  - The US HPV and OECD HPV programs provided:
    - Aquatic toxicity data for approx. 160 substances (70% accepted)
  - Persistence data for approx. 140 substances (90% accepted)
  - Bioaccumulation data for approx. 10 substances (90% accepted)

Data Preference for P B iT Profiles

Preference	P	B	iT
Higher	Experimental		
Medium	Analogue / Groupings / Scientific rationale		
Lower	Modelled (QSAR)		

Stakeholder Submission of Data

- June 2004, Canada launched an 18 month voluntary challenge to industrial stakeholders and interested parties to submit experimental study or other information that could help refine categorization decisions
- We received approx 20 larger data submissions for consideration and more than 400 individual studies addressing P, B or aquatic toxicity
- Approx. 20 submissions have been received covering the human health aspects of categorization

Categorization
Human Health Aspects

- Early recognition that legislative construct for categorization would not identify all priorities from human health perspective
- Persistence and Bioaccumulation not key determinants of potential to harm human health for all types of substances
- Other properties more relevant for some chemicals (e.g., reactive compounds)
- Simple and Complex Exposure and Hazard Tools developed to identify health priorities

The Simple Exposure Tool - SimET

- SimET is a relative ranking tool by which all substances on the Domestic Substances List have been "binned"
- Maximal use of the limited, comparable data for all 23,000 DSL compounds
  - Prevents bias to data-rich compounds
- Based on three different lines of evidence, derived from the limited information provided for all substances on the DSL:
  - quantity (estimated annual quantity of use, Q),
  - number of submitters (S)
  - use (sum of normalized expert ranked use codes, (U), reflecting two workshops
- Limited expert judgement

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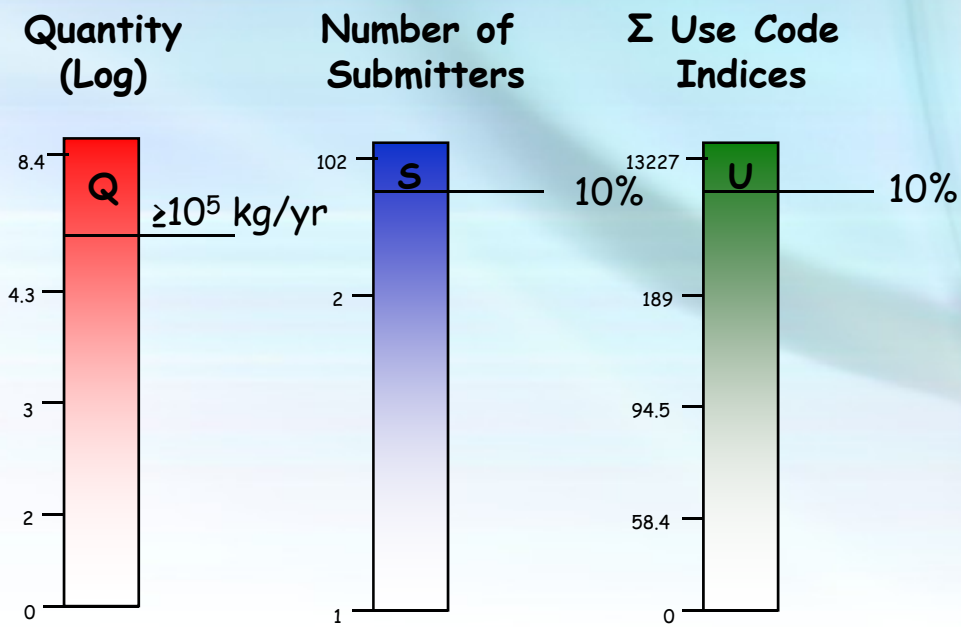
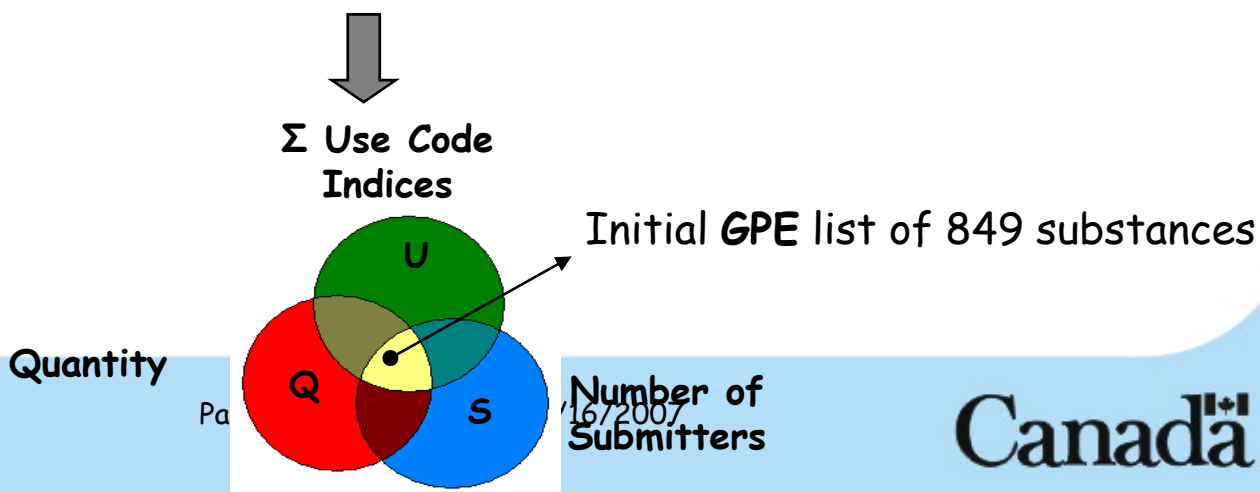
Simple Exposure Tool (SimET): Relative Ranking for all DSL substances





Government of Canada Gouvernement du Canada
Canada

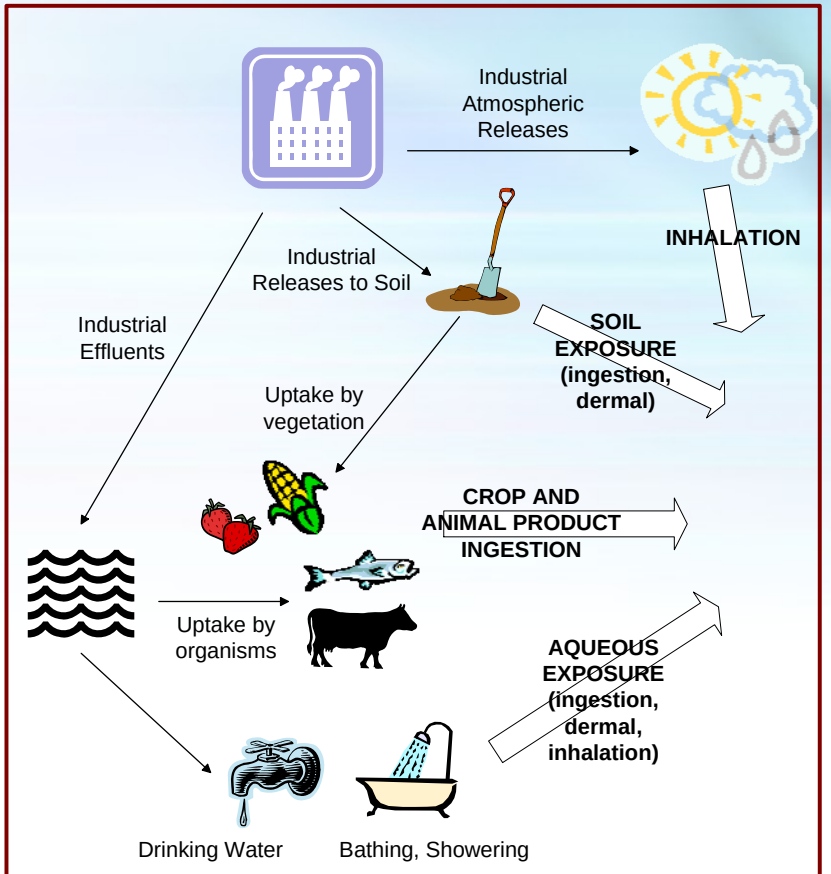
Criteria for Greatest, Intermediate & Lowest Potential for Exposure (GPE, IPE & LPE)

	Quantity (kg/year)	Number of Submitters	Sum of the Expert Ranked Use Code Indices
GPE	> 100 000	Top 10%	Top 10%
IPE	> 10 000	n.a.	Top 30%
LPE	All	All	All

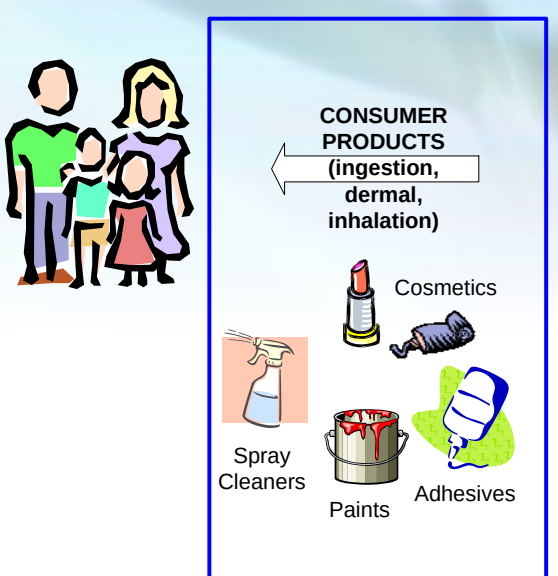
ComET – the Complex Exposure Tool

- Provides plausible upper bound quantitative estimates of combined consumer (nearfield) & multimedia environmental (farfield) exposure by duration and age group, taking into account:
  - "Sentinel" product scenarios
    - Uses for a particular chemical likely to result in highest exposure
    - Based on upper bound of proportion used for generic function in a type of product
      - E.g., "solvent in paints", "polymers in adhesives"
  - Concentrations in environmental media of human exposure estimated based on extension of fugacity modelling

The Complex Exposure Tool (ComET)



ComET - Exposure to man from the environment



ComET - Near-field exposures

The Simple Hazard Tool - SimHaz

- Applied to entire DSL
- Defines high or low hazard from classifications/assessments of other agencies based on weight of evidence
- Appropriate assessments selected based on comprehensiveness of review, peer review process, etc.

SimHaz Tool

- High Hazard Lists/Endpoints
  - Cancer (IARC, EU, HC, US EPA etc.)
  - Genotoxicity (EU)
  - Developmental Toxicity (EU)
  - Reproductive Toxicity (EU)
- Low Hazard Lists
  - PMRA 4a/US EPA 4a
  - OECD Low Concern

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SimHaz Tool
Strengths and Limitations

- Strengths
  - Efficient
    - Takes advantage of critical review of others
  - Consistency
    - Assessments/classifications internationally
- Limitations
  - Bias towards data-rich substances

ComHaz Tool

- Hierarchical approach to consideration of
  - Multiple endpoints relevant to characterization of hazard
  - Sources of relevant information

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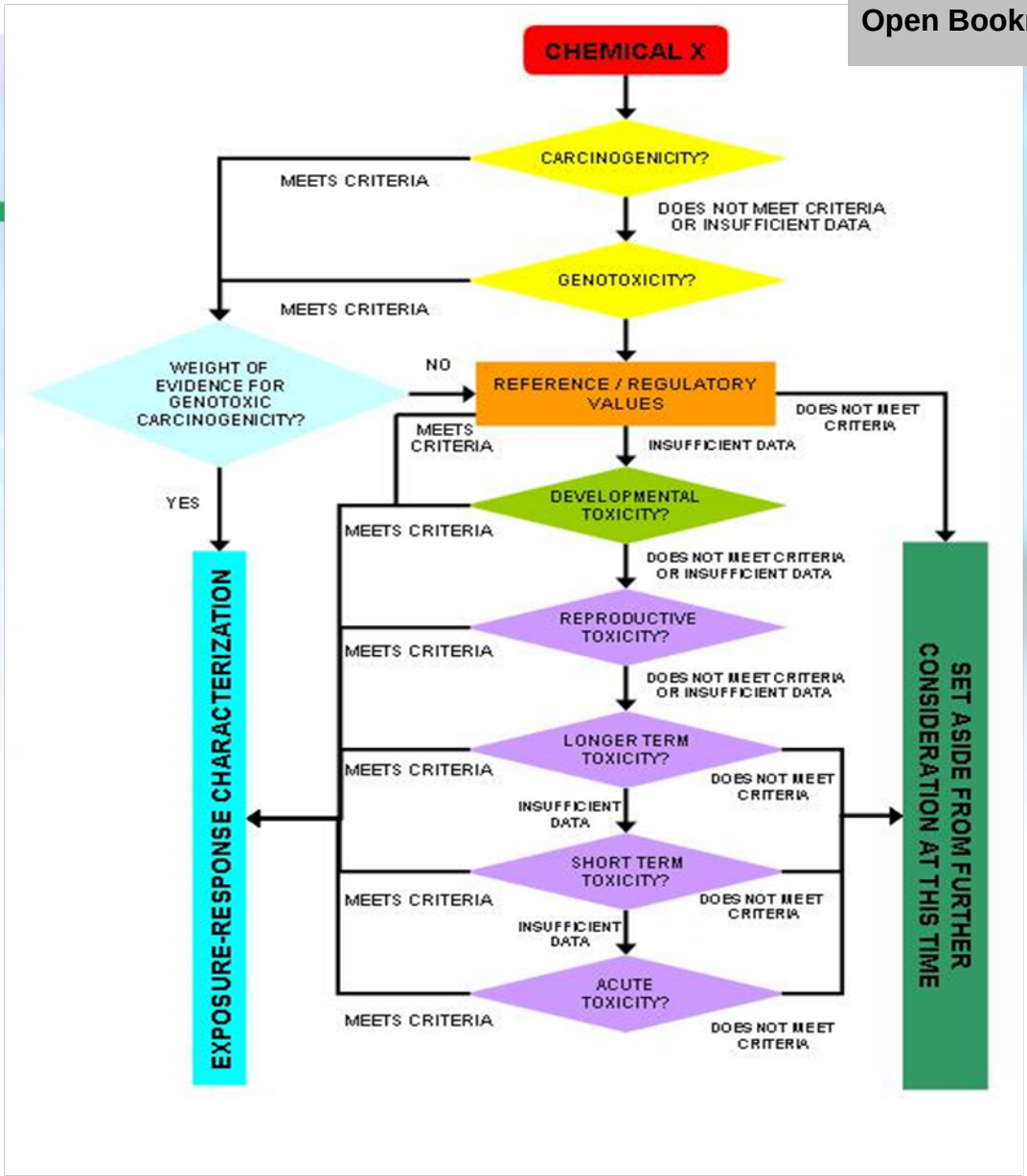
ComHaz Hierarchy



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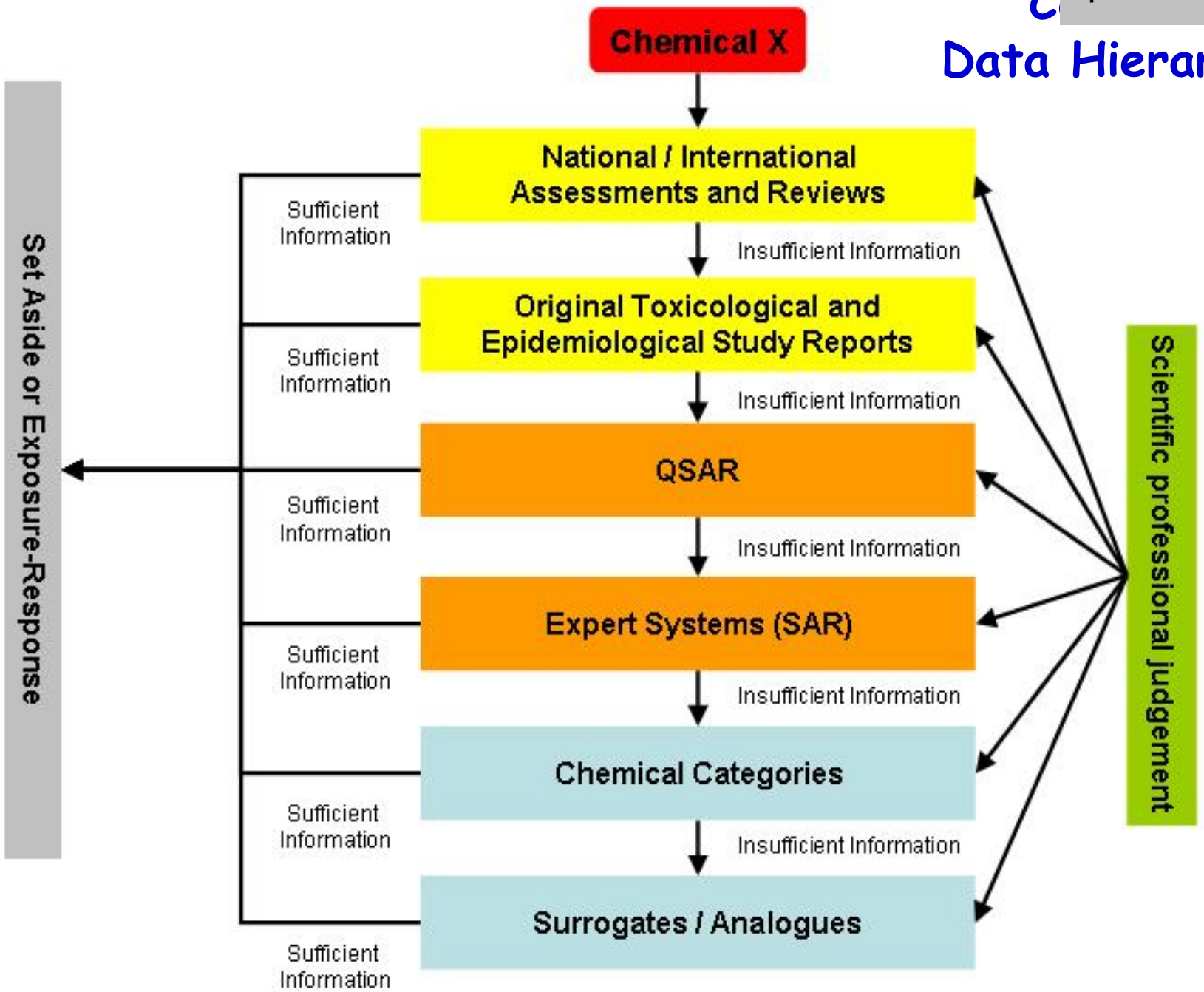
Data Hierarchy



ComHaz Tool – Endpoint-specific Criteria

Endpoint	Information Source	Criteria
Cancer	Data or (Q)SAR	1) Positive evidence 2) Weight of evidence
Genotoxicity	Data or (Q)SAR	1) Positive evidence 2) Weight of evidence
Regulatory/Reference Value	International & National Assessments	Ref Value ≤ 0.1 mg/kg bw/day
Developmental Toxicity	Data	NO(A)EL ≤ 90 mg/kg bw/day
	(Q)SAR	Positive Prediction
Reproductive Toxicity	Data	NO(A)EL ≤ 10 mg/kg bw/day
Longer Term Toxicity	Data or (Q)SAR	NO(A)EL ≤ 10 mg/kg bw/day
Short Term Toxicity	Data	NO(A)EL ≤ 30 mg/kg bw/day
Acute Toxicity	Data or (Q)SAR	LD_{50} ≤ 500 mg/kg bw

ComHaz Tool
Weight of Evidence (WoE)

Cancer/Genotoxicity

- First endpoints in the hierarchy and those for which capture rate is highest
- Confidence in (Q)SAR is greatest for these endpoints
  - Larger more diverse training sets (e.g., simple screening assays such as Ames test, specific sex/species combinations for cancer)
  - Potential for combining relevant endpoints
  - Relevance to specific modes of action
- Consistency with approach for PSL assessments that genotoxic carcinogenicity is critical endpoint

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ComHaz Tool
WoE Cancer/Genotoxicity

- Incorporates separate lines of evidence, with differential weighting, including empirical data, QSAR/SAR results (CASETOX, TOPKAT, DEREK) & information from analogs
- Relative weighting of individual cancer & genotoxicity results, based on internal experience/expert consultation
  - For cancer, route of administration and numbers of species/sexes
  - For genotoxicity, predictive strength of various assays (i.e., in vivo vs. in vitro mutagenicity/clastogenicity assays > DNA damage > Indicator tests)

ComHaz Tool
Strengths and Limitations

- Strengths
  - Health protective
  - Comprehensive
  - High confidence in "set asides"
  - No bias towards data rich substances
  - Designed for high throughput
  - Takes advantage of critical reviews of others
  - Significant contribution of QSAR component to priority setting
  - External input, consultation, peer review
- Limitations
  - Resource intensive

Results of Categorization

- Categorization/Prioritization completed in September 2006
- Identified priorities for further work/action based on concern for:
  - Environment
  - Human Health
  - Environment & Human Health
- Of the 23,000 substances on the DSL, 4300 identified as priorities
  - 4000 met the categorization criteria
  - 300 warrant further attention from a human health perspective
- Set the stage for subsequent screening assessments

The Way Forward
The Chemicals Management Plan

- Following completion of categorization, Canada's Chemicals Management Plan (CMP) was introduced
- The CMP builds on categorization initiative to improve protection against hazardous substances
- Includes new, proactive measures to ensure chemical substances are managed properly

Key Objectives of the Chemicals Management Plan

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- Significantly strengthen the existing substances regime:
  - Categorization established a new information baseline that sets clear priorities for action that are science based
- Integrate government activities:
  - Strengthen CEPA's coordination with other federal statutes, including: *Hazardous Products Act*, *Food & Drugs Act*, and *Pest Control Products Act*

Key Objectives of the Chemicals Management Plan (cont'd)

- Establish government accountability:
  - Enhanced monitoring and surveillance activities to identify priorities and measure effectiveness of regulatory actions
  - Increased research activities to ensure that action is informed by best available science
  - Enhanced risk communications to Canadians
  - Public web site to ensure consistent access to information
  - A cyclical update of the Domestic Substances List that will require industry to report on use and volume of substances on the Canadian market

- Strengthen industry's role in proactively identifying and safely managing risks associated with chemicals they produce and use

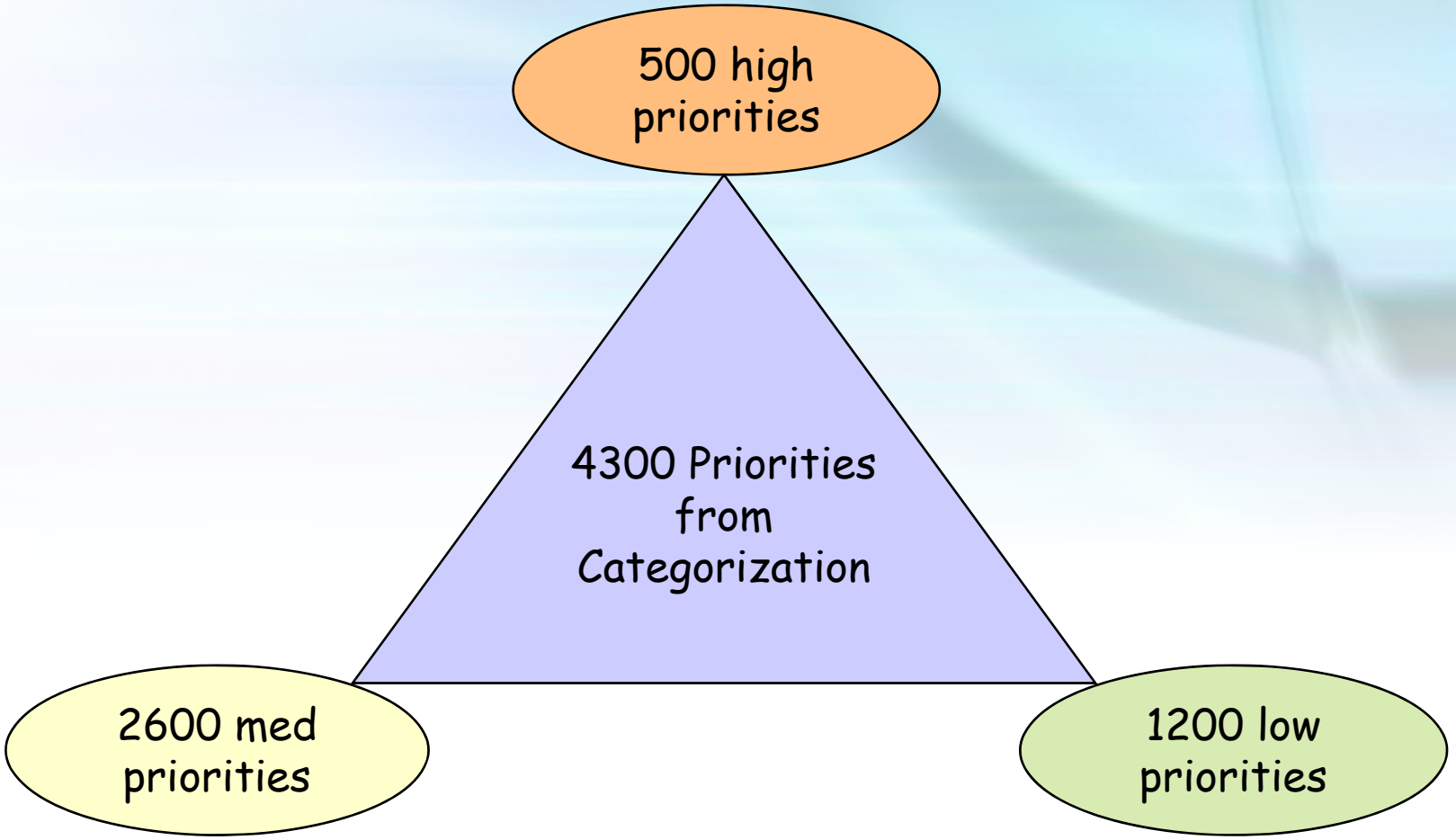
Furthering the Assessment of Existing Substances under the Chemicals Management Plan

- CMP directed that substances of highest concern of those identified in categorization be addressed
- Need to further prioritize substances identified during categorization exercise
- "Focus attention where attention is due"
- Capitalized on information gathered during categorization process

Prioritizing the Priorities

- Considerations for the following round of priority setting and upcoming actions :
  - The degree of hazard/risk
  - Commercial activity in Canada
  - Existing/ongoing risk assessment and risk management activities
  - Opportunities to engage internationally and "share the work" for a global issue

Prioritizing the Priorities



Low Hazard, Low Risk Substances

- A number of PiTeco or BiTeco substances identified through categorization have a low potential for risk
- Rapid screening employed for risk assessment used a worst-case scenario model to confirm the likelihood that a substance may not cause ecological harm
- Results were released for public comment on June 23, 2007
  - 1066 substances were subject to this approach
  - 754 substances were found to be "not toxic" to the environment according the definition in CEPA 1999
  - The government proposes to include the substances in the future inventory update to validate assumptions, conducting monitoring where appropriate and revisit some of the substances as part of assessments of "families of chemicals" at a later date
  - 312 substances require further assessment and re-prioritized into the group of medium priority substances

Medium priority substances

- The medium priority substances are expected to be addressed by 2020
- International programs, support for the research and monitoring community, and development and update of a cyclical inventory update will help the Government set priorities
- In addition, we will work with industrial sectors to negotiate and implement performance agreements

High Priority Substances

- High priorities for action included substances
  - That met each of the ecological categorization criteria (persistence (P), bioaccumulation (B) and inherent toxicity to aquatic organisms (iT), and believed to be in commerce in Canada and/or;
  - That met the criteria for greatest or intermediate potential for exposure (GPE or IPE) and were identified as posing a high hazard to human health (i.e., classified by another agency on the basis of carcinogenicity, mutagenicity, developmental toxicity or reproductive toxicity as identified by SimHaz Tool)

High Priorities

- Within the Chemicals Management Plan, the top priorities (identified through categorization) are addressed through 4 components:
  - Significant New Activity (SNAcs) for substances believed to not be in commerce
  - Petroleum Sector Stream – a focused sectoral approach
  - Substances that are already in the assessment or management stream
  - Challenge Program for substances believed to be in commerce (~ 200 substances)

Restrictions on Re-introductions and New Uses (SNAcs)

- Re-introduction of 148 high-hazard substances from categorization not currently in use in Canada controlled through the Significant New Activity (SNAc) provisions of the New Substances Program

- SNAc notice requires industry to provide data o be reviewed by Environment Canada and Health Canada before the substance can be re-introduced into Canada

Petroleum Sector Stream

- High priority petroleum substances were set aside from the Challenge because of the large number that are primarily or exclusively related to the petroleum sector
- Most are complex mixtures that may need to be considered differently from discrete substances
- Will be assessed within three years, with control measures, if appropriate, within CEPA timelines
- The Government is currently developing a work plan and will consult with stakeholders

The Challenge Program

- The Chemicals Management Plan relies on strong stewardship from the Canadian industry
- Challenge to stakeholders to provide data to:
  - Improve, where possible, PBiT data
  - Identify industrial best practices in order to set benchmarks for risk management and product stewardship
  - Collect environmental release, exposure, substance and/or product use information
  - Information about how it is managing these substances
- The absence of information will not preclude Government from taking action that safeguards human health and the environment

Why Canada is using a Challenge initiative to deal with the 200 priorities for action

The Ministers consider:

- Evidence that a substance is both Persistent and Bioaccumulative, when combined with evidence of toxicity and release into the environment, can lead to harmful ecological impacts. This indicates that the substance meets at least one of the criteria for "toxic" to the environment in CEPA 1999
- Evidence that a substance for which the critical health effect is assumed to have no threshold – i.e. a genotoxic carcinogen – it is assumed that there is a probability of harm to human health at any level of exposure, and therefore indicates that the substance meets the criteria for "toxic" to human health in CEPA 1999
- Evidence that a substance exhibits carcinogenicity, mutagenicity, developmental toxicity, or reproductive toxicity, and a high likelihood of exposure to individuals in Canada, indicates that the substance meets the criteria for "toxic" to human health in CEPA 1999

What is the "Challenge"?

- Publication, in batches of 15-30 substances every three months, a substance profile for industry and other stakeholders to comment on and provide any additional information in their possession (mandatory and non-mandatory responding)
- Stakeholders will have six months to comment on the profiles and provide requested information
- All challenge substances to be released within 3 years
- Government scientists will have a maximum of 6 months to review the information provided. The Government of Canada will then decide what actions are to be taken through an expedited application of CEPA (1999)
- Risk management actions for all substances will be implemented in accordance with the CEPA Process

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Objectives of the Substance Profile Documents

- Show stakeholders what we know about the substance.
- Justify, within the context of the Challenge, the basis for taking actions on a substance that was identified as a high concern through the categorization activities.
- Identify opportunities to submit information to support the activities taking place under the Chemicals Management Plan.

Screening Assessments

- For substances identified in the Challenge, screening level risk assessments are being prepared to address
  - Ecological aspects
  - Human health aspects
  - Ecological and Human health aspects

- Depending on the basis under which they were considered priorities for further work

Ecological Screening Assessments

Substance Profile

↓

Screening Assessment

As appropriate:

- Refine phys-chem properties
- Revise decisions on persistence, bioaccumulation and long-range transport
- Refine information on possible toxic effects, including information for non-aquatic organisms, where available
- Refine information on commercial activities and other potential sources of formation or release
- Refine summary of key uncertainties

Human Health Screening Assessments

- Generally, national/international classifications will be used as the basis for characterizing the hazard of these substances for the screening assessment
  - incorporates weight of evidence based decisions of these agencies
  - ensures consistency
- Consideration also of other health effects of potential concern beyond the basis for the classification based on evaluation of available information (including recently published peer-reviewed studies) in a screening context
- Characterization of population exposure and key sources
- Discussion of uncertainties

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Prioritization & Assessment Past & Future

- Capitalize on past efforts to move forward on these substances through strengthened partnerships inside and outside the federal government to ensure the most efficient and effective protection of Canadians and their environment
  - Program expertise
  - Experienced stakeholder engagement relationships
  - Targeted legislative design

Contact Information

- Chemical Substances Website: http://www.chemicalsubstances.gc.ca
- CD-ROMS with results of DSL Categorization are available upon request.

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Thank you!

1 June 2009

General Approach for Prioritisation of Substances of Very High Concern (SVHCs) for Inclusion in the List of Substances Subject to Authorisation

Document developed in the context of ECHA's first Recommendation for the inclusion of substances in Annex XIV

1 Introduction

Pursuant to Article 58(3) of the Regulation (EC) No 1907/2006[1] (REACH), whenever a decision is taken to include substances referred to in Article 57 of REACH in Annex XIV, priority shall normally be given to substances with *PBT or vPvB properties, or wide dispersive use, or high volumes.* As indicated in recital 78 of REACH "*the Agency should provide advice on the prioritisation of substances to be made subject to the authorisation procedure, to ensure that decisions reflect the needs of society as well as scientific knowledge and developments*".

This document describes the general approach taken by ECHA for prioritising the substances that are listed on the present list[2] of candidate substances for inclusion in Annex XIV.

ECHA decided to use a pragmatic approach for the priority setting of the substances on the current 'candidate list', which mainly relies on the legal criteria provided for in Article 58(3) of REACH. The approach to assess the criteria is a qualitative, where possible semi-quantitative, evaluation resulting in an overall conclusion on the priority of a substance. The conclusion based on the criteria of Article 58(3) may, however, be superseded by additional considerations indicating that prioritisation of a substance for inclusion in Annex XIV would from a regulatory point of view either be appropriate or not be appropriate.

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[1] Corrigendum to Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (OJ L 396, 30.12.2006); amended by Council Regulation (EC) No 1354/2007 of 15 November 2007 adapting Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), by reason of the accession of Bulgaria and Romania (OJ L 304, 22.11.2007, p. 1).

[2] Candidate List of Substances of Very High Concern for authorisation: http://echa.europa.eu/chem_data/candidate_list_table_en.asp

It should be noted that a conclusion to not give priority to the inclusion of a particular candidate substance in Annex XIV is only relevant for the respective priority setting operation in which this conclusion has been drawn. In subsequent priority setting operations, this substance may be re-considered for inclusion in Annex XIV together with all other substances on the candidate list which are not already included in Annex XIV.

Although the present low number of substances on the candidate list does not necessarily warrant an advanced priority setting mechanism capable of differentiating and prioritising among many substances on the basis of detailed information on properties, uses and volumes of a substance, the chosen approach is developed to ensure a coherent priority setting for substances, taking into account the fact that the list will likely grow as a result of regular future updates. The approach, while it can be kept consistent with regard to the criteria applied, could be further enhanced and refined when more data on the individual substances becomes available (e.g. due to more elaborated Annex XV dossiers and/or the increasing availability of registration dossiers).

2 Outline of the priority setting approach

The information used in accordance with the criteria of REACH Article 58(3) refers to (eco)toxicological properties (hazard), to potential for release and exposure as well as to volumes supplied. This information, in particular when assessed in combination, could be seen as a proxy for potential risk to human health or the environment (i.e. the higher the hazard, the volume used and the potential for release of a substance, the higher its potential risk and thereby its priority). Hence the approach outlined in the following can be considered as risk-based. It should be noted that the actual assessment of the risks to human health and/or the environment should be included by the applicant when submitting the chemical safety report as part of the application for authorisation.

2.1 Prioritisation criteria and their parameterisation

According to Article 58(3) priority for inclusion in Annex XIV shall normally be given to substances with

a) PBT or vPvB properties; or
b) wide dispersive use; or
c) high volumes.

The term “normally” implies that the criteria mentioned in Article 58(3) do not need to be seen as exclusive, allowing other considerations to be taken into account which may warrant a higher or lower priority for a substance, in particular in relation to the regulatory effectiveness of selecting the substance.

Given that the listing of the criteria is connected by the term *‘or’*, this means that meeting one criterion could in principle be sufficient to prioritise a substance for inclusion in Annex XIV. However, in order to allow further differentiation the approach actually followed for prioritising the candidate substances for inclusion in Annex XIV also considers how many of these three criteria are met by the candidate substance concerned and to what degree.

Therefore, it is necessary to consider the rationale of the criteria and to identify and select parameters by which the rationale of the criteria can be reflected. The following parameterisation of the above criteria *a), b) & c)* has been used for prioritisation.

a) PBT or vPvB properties

Substances that have been identified as having PBT or vPvB properties under Article 57 (d), (e) or (f) meet criterion *a)* on *'PBT or vPvB properties'* and may be prioritised for inclusion in Annex XIV. However, not fulfilling criterion *a)* does not preclude that CMR properties of substances meeting criteria *b) 'wide dispersive use'* and/or *c) 'high volumes'* can be considered in the prioritisation[3].

b) Wide dispersive use

The term "wide dispersive use" is explained in Chapter R.16.2.1.6 of the Guidance on Information Requirements and Chemical Safety Assessment as follows: *"Wide dispersive use refers to many small point sources or diffuse release by for instance the public at large or sources like traffic. … Wide dispersive use can relate to both indoor and outdoor use"*. In the Technical Guidance Document for Risk Assessment of new and existing substances and biocides (2003, Chapter 5) this term is defined as follows: "*Wide dispersive use refers to activities which deliver uncontrolled exposure. Examples relevant for occupational exposure: Painting with paints; spraying of pesticides. Examples relevant for environmental/consumer exposure: Use of detergents, cosmetics, disinfectants, household paints."* In addition, the ECETOC Report No. 93 on Targeted Risk Assessment (Appendix B) states: *"A substance marketed for wide dispersive use is likely to reach consumers, and it can be assumed that such a substance will be emitted into the environment for 100% during or after use."*

Wide dispersive uses are hence characterised by use(s) of a substance on its own, in a preparation or in an article at many places that may result in not insignificant releases and exposure to a considerable part of the population (workers, consumers, general public) and/or the environment. This means that uses taking place at many places, which however do not result in significant releases of a substance, may be considered only as 'widespread' but not as 'wide dispersive'.

In general, consumer use can be considered as wide dispersive if it can be reasonably assumed that this use results in non-negligible releases. Professional use can be wide dispersive as well if it takes place at many sites and is carried out by many workers and if it cannot be excluded that releases are negligible.

To assess the criterion *'wide dispersive use'*, it might be useful to consider also potential releases and exposure from uses other than 'wide dispersive', as not only wide dispersive uses may result in releases and contributions to exposure. However, for the current prioritisation no information was available allowing this aspect to be taken into account.

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[3] For the present prioritisation, a differentiation was only made between PBT or vPvB properties and CMR properties of the candidate substances. If in future the candidate list will become substantially longer, it may be necessary to further differentiate between substances on the basis of their intrinsic properties (i.e. PBT or vPvB properties versus C, M or R properties) if a similar priority results from the degree of fulfilment of criteria *b)* and *c).*

Depending on the information available, as many as possible of the following parameters are used as indicators to assess whether a use (and the resulting release) should be considered 'wide dispersive' and to get an at least qualitative indication on the degree of its 'dispersiveness':

- Tonnage going to the use.
- The complexity of the supply chain and the number of actors in the chain. In how many settings/locations does the use take place? What are the typical sizes of these settings?
- In which form is the substance placed on the market (e.g. as such, as part of a preparation, in/on an article)?
- Can the substance be released (and to which extent) during the service life of an article or a preparation (e.g. paints, adhesives, detergents) or is it transformed (thereby loosing its hazardous properties) or incorporated into a matrix (e.g. polymer) in a way preventing release?
- Information on operational conditions and risk management measures.
- Information on whether there is occupational exposure (quantitative or qualitative; e.g. approximate number of exposed workers, information on releases to the working environment, occupational exposure concentrations, health effects, OELs).
- Information whether there is consumer exposure (quantitative or qualitative; e.g. possibility of consumer use, information on consumer exposure, health effects, limit values).
- Releases to the environment (mainly for PBTs/vPvBs; e.g. t/y to the different compartments air, water, soil).
- Possibility of releases during the waste phase.
- Monitoring information for a substance in environmental compartments such as water, sediment, soil or in biota.

The parameters listed above are used in a weight of evidence approach. The priority of a substance increases with the portion of its uses (respectively the tonnage supplied to these uses) identified as wide-dispersive and the (estimated) released volumes from those wide dispersive uses. Likewise, no or lesser priority will be given when no significant releases occur from these uses or when the releases are comparatively low. As regards the releases, the focus is normally on environmental releases for substances with PBT properties and on releases leading to potential human exposure for CMR substances.

c) High volumes

The annual volume supplied in the EU to uses not exempted from the authorisation requirement[4] is taken as parameter for this criterion, i.e.:

[4] Appendix 1 to this document provides a list of uses specifically exempted from the authorisation requirement. Exemptions relevant in the context of the present prioritisation exercise are, for example, uses as on-site isolated intermediates or as transported intermediates, uses in biocidal products and uses in scientific research and development.

Volume supplied =
(Manufacture + Import) – (Export + supply to uses exempted from authorisation)

The total annual tonnages are considered as:

Low volumes, if	<10 t/y;
Relatively low volumes, if	10 - <100 t/y;
Relatively high volumes, if	100 - <1,000 t/y;
High volumes, if	1,000 - <10,000 t/y;
Very high volumes, if	>10,000 t/y.

Priority increases with increasing volume.

Note that the 'volume' criterion is considered not to be met if:

- There are no identified uses of the substance in the EU;
- There are no uses identified that are not exempted from the authorisation requirement.

No use of a substance or no use in the scope of the Authorisation Title of REACH implies logically that the criterion 'wide dispersive use' is not fulfilled for this substance.

Not prioritising a substance that has no identified uses in the EU will help to prevent developing Annex XIV into a list of obsolete substances.

2.2 Additional considerations for prioritising a candidate substance

In arriving at the overall conclusion on the priority of a substance, the 'regulatory effectiveness' of including the substance into the authorisation process is also taken into account. Situations may for instance occur where inclusion in Annex XIV will only require regulatory efforts but most likely will not result in benefits for human health or the environment. For example:

- All identified uses are subject to specific Community legislation imposing minimum requirements relating to the protection of human health or the environment ensuring that risks are properly controlled.
- All or most known uses can easily be replaced by another 'form' of the substance with a similar (or even worse) hazard profile, which is not on the candidate list (e.g. one metal salt on the candidate list can be replaced by another salt of the same metal with the same hazard profile, but this salt is not on the candidate list).
- Uses have been identified but the resulting releases are insignificant as such or insignificant compared to releases resulting from natural sources and/or uses not in the scope of the Authorisation Title of REACH.

In the first case, risks are already properly controlled by other Community legislation, and in the second case the authorisation requirement can easily be circumvented by replacement of the substance subjected to the authorisation requirement by the other 'form' of the substance not requiring authorisation.

Regarding the second case, a grouping approach could be considered[5] in order to prevent simple replacement of a substance that will be subjected to authorisation by another 'form' of the substance.

2.3 Conclusion on the priority of a candidate substance

The three prioritisation criteria related to the intrinsic properties of a candidate substance, the nature of its uses and its volume supplied to uses in the scope of the Authorisation Title of REACH are assessed together in a weight of evidence approach in a qualitative, where possible semi-quantitative manner, resulting in an overall conclusion on the priority of the substance. The number of criteria met and the extent to which the criteria are fulfilled (i.e. the higher the rating of the intrinsic properties, the more wide dispersive the uses and the higher the volumes not exempted from Authorisation) are important factors in deciding whether or not to prioritise a substance.

However, in arriving at the final conclusion on the priority of a substance the additional considerations regarding 'regulatory effectiveness' outlined in section 2.2 may also play an important role.

[5] Whether grouping of substances with similar properties is required in order to ensure the efficacy of authorisation in terms of the envisaged benefits for human health and/or the environment should already be considered in the planning-phase of Annex XV dossiers for identification of substances as SVHCs. A prerequisite for being able to group substances with the objective to subject them as a group to the authorisation requirement is their prior identification as SVHCs and inclusion in the candidate list.

APPENDIX 1: USES EXEMPTED FROM AUTHORISATION

On-site isolated intermediates and transported isolated intermediates {Art. 2(8b)}.
Use in medicinal products for human or veterinary use within the scope of Regulation (EC) No 726/2004, Directive 2001/82/EC and Directive 2001/83/EC {Art. 2(5a)}.
Use in food or feedingstuffs according to Regulation (EC) No 178/2002 including use as a food additive in foodstuffs within the scope of Council Directive 89/107/EEC, as a flavouring in foodstuffs within the scope of Council Directive 88/388/EEC and Commission Decision 1999/217/EC or on foodstuffs drawn up in application of Regulation (EC) No 2232/96, as an additive in feedingstuffs within the scope of Regulation (EC) No 1831/2003 and in animal nutrition within the scope of Council Directive 82/471/EEC {Art. 2(5b)}.
Use in scientific research and development {Art. 56(3)}.
Use on plant protection products within the scope of Council Directive 91/414/EEC {Art. 56(4a)}.
Use in biocidal products within the scope of Directive 98/8/EC {Art. 56(4b)}.
Use as motor fuels covered by Directive 98/70/EC {Art. 56(4c)}.
Use as fuel in mobile or fixed combustion plants of mineral oil products and use of fuels in closed systems {Art. 56(4d)}.
Use in cosmetic products within the scope of Council Directive 76/768/EEC (this exemption applies to substances listed on Annex XIV on the basis of their hazard to human health only) {Art. 56(5a)}.
Use in food contact materials within the scope of Regulation (EC) No 1935/2004 (this exemption applies to substances listed on Annex XIV on the basis of their hazard to human health only) {Art. 56(5b)}.
Use of substances when present in preparations below a concentration limit of 0.1% by weight. This applies only to substances listed in Annex XIV on the basis of being persistent, bioaccumulative and toxic (PBT) as defined by Art. 57(d), very persistent and very bioaccumulative (vPvB) as defined by Art. 57(e), or listed in Annex XIV on the basis that there is scientific evidence of probable serious effects to human health or the environment which give an equivalent level of concern to substances with PBT or vPvB properties, or an equivalent level of concern to substances classified as carcinogenic, mutagenic or toxic for reproduction (CMR) category 1 and 2, as defined by Art. 57(f) {Art. 56(6a)}.
Use of substances when present in preparations below the lowest concentration limits specified in Directive 1999/45/EC or in Annex I to Council Directive 67/548/EEC which results in the classification of the preparation as dangerous. This applies only to substance listed in Annex XIV on the basis of their classification as CMR category 1 and 2 {Art. 56(6b)}.

Green Ribbon Science Panel

Topic #1 --- Chemical Identification & Prioritization

Statute:

- DTSC shall adopt regulations to establish a process to identify and prioritize those chemicals or chemical ingredients in consumer products that may be considered as being a chemical of concern.

- The regulations shall establish an identification and prioritization process that includes, but is not limited to, all of the following considerations: (1) the volume of the chemical in commerce in this state; (2) the potential for exposure to the chemical in a consumer product; and (3) potential effects on sensitive subpopulations, including infants and children.

- DTSC shall develop criteria by which chemicals and their alternatives may be evaluated. These criteria shall include, but not be limited to, the traits, characteristics, and endpoints that are included in the clearinghouse data.

- DTSC shall reference and use, to the maximum extent feasible, available information from other nations, governments, and authoritative bodies that have undertaken similar chemical prioritization processes --- DTSC is not required to reference and use only the available information from these sources.

- DTSC shall adopt regulations that establish a process for evaluating chemicals of concern in consumer products, and their potential alternatives, to determine how best to limit exposure or to reduce the level of hazard posed by a chemical of concern.

See Attachment 1-1 for complete statutes that provide the mandate, authorities and scope for these regulations.

LIST OF ATTACHMENTS:

1-1 Statutes
1-2 Authoritative Bodies Lists Examples
1-3 Prioritization Hazard & Exposure Factors Example (modeled on draft regulations)
1-4 Prioritization Exposure Factors Examples
1-5 Ranking Formula Approach Examples
1-6 USEPA Design for the Environment AA Criteria for Hazard Evaluation (draft)
1-7 Washington State Chemical Prioritization (presentation by Alex Stone)
1-8 Reliable Information Definition Examples (modeled on draft regulations)
1-9 OECD Guidance on Data Quality

Question #1A: **Should there be multiple chemicals lists?**

(i) Should there be a single chemicals list (e.g., “Chemicals of Concern”), or two hierarchical chemicals lists (e.g., “Chemicals of Concern” and “Priority Chemicals”)?

(ii) If there are two lists, should they be developed concurrently or sequentially?

(iii) Other ideas?

Question #1B: **What criteria/process should be used to place chemicals on the list or lists?**

(i) Should one or both lists be a compilation of all chemicals listed by other specified authoritative bodies --- if so, which authoritative bodies?

(ii) Should one or both lists be a compilation of all chemicals that exhibit one or more specified hazard traits --- if so, should this be all of the OEHHA-listed hazard traits or a subset thereof (if the latter, which ones)?

(iii) What chemical hazard factors should be used to prioritize chemicals?

(iv) Should exposure potential factors also be used to prioritize chemicals --- if so, what factors?

(v) If there are two chemicals lists, what chemical hazard factors and what (if any) exposure potential factors should be used for each list?

(vi) In assessing a chemical's threat of adverse impact associated with each identified prioritization factor:

- Should there be a threshold established for each factor --- if so, how should the thresholds be determined?
- Should the prioritization evaluation be based on the chemical's individual threat of adverse impact, or the chemical's contribution to cumulative adverse impacts? If the latter, how should this be evaluated?

(vii) Should one or both lists be developed using a ranking formula applied to the identified prioritization factors? If so:

- How should the ranking formula be designed?
- Should the factors be weighted? If so, how should the weights be determined?
- Should numerical values (e.g., 1 to 10) or high-medium-low be used to score each chemical for each factor, and to assign an overall score? How should the scores be determined?

(viii) Should one or both lists also include "known bad actors"? If so:

- What are these and/or how should they be determined?
- Should these include chemicals that present end-of-life management or cost concerns?
- Should these include chemicals considered likely to present exposures to sensitive subpopulations?

(ix) Other ideas?

Question #1C: Should the chemical list(s) be limited to specified chemicals or chemical categories during early implementation years?

(i) Should there be a scope limitation, or not?
The remaining questions below assume that there will be a scope limitation.

(ii) If there are two chemicals lists, should the scope limitation apply to one or both lists?

(iii) What criteria should be used for the scope limitation?

- Hazard traits --- which ones?
- Presence on other authoritative bodies' lists --- which ones?
- Use in specified types of products --- which ones?
- End-of-life management and cost concerns --- which ones (e.g., MSW landfill disposal bans, chemicals showing up in state and local waste streams)?
- Other ideas?

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Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development **02-Jun-2010**

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ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

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GUIDANCE MANUAL FOR THE TESTING OF MANUFACTURED NANOMATERIALS: OECD's SPONSORSHIP PROGRAMME; FIRST REVISION

This is the first revision of the Guidance Manual for the Testing of Manufactured Nanomaterials first published in 2009 [ENV/JM/MONO(2009)20]. This document is intended to support the testing of manufactured nanomaterials, undertaken in the context of OECD's Sponsorship Programme.
It was always envisaged that this would be a living document and subsequent revisions will be made available in the future.

English - Or. English

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OECD Environment, Health and Safety Publications
Series on the Safety of Manufactured Nanomaterials

No. 25

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IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among UNEP, ILO, FAO, WHO, UNIDO, UNITAR and OECD

Environment Directorate
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No. 21, *Report of the Workshop on Risk Assessment of Manufactured Nanomaterials in a Regulatory Context (2010)*

No. 22, *OECD Programme on the Safety of Manufactured Nanomaterials 2009-2012: Operational Plans of the Projects (2010)*

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No. 24, *Preliminary Guidance Notes on Sample Preparation and Dosimetry for the Safety of Manufactured Nanomaterials (2010)*

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ABOUT THE OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 31 industrialised countries in North America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in ten different series: Testing and Assessment; Good Laboratory Practice and Compliance Monitoring; Pesticides and Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and the Safety of Manufactured Nanomaterials. More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (http://www.oecd.org/ehs).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organisations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The participating organisations are FAO, ILO, OECD, UNEP, UNIDO, UNITAR and WHO. The World Bank and UNDP are observers. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

The OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology (the Joint Meeting) held a Special Session on the Potential Implications of Manufactured Nanomaterials for Human Health and Environmental Safety (June 2005). This was the first opportunity for OECD member countries, together with observers and invited experts, to begin to identify human health and environmental safety related aspects of manufactured nanomaterials. The scope of this session was intended to address the chemicals sector.

As a follow-up, the Joint Meeting decided to hold a Workshop on the Safety of Manufactured Nanomaterials in December 2005, in Washington, D.C. The main objective was to determine the "state of the art" for the safety assessment of manufactured nanomaterials with a particular focus on identifying future needs for risk assessment within a regulatory context.

Based on the conclusions and recommendations of the Workshop [ENV/JM/MONO(2006)19] it was recognised as essential to ensure the efficient assessment of manufactured nanomaterials so as to avoid adverse effects from the use of these materials in the short, medium and longer term. With this in mind, the OECD Council established the OECD Working Party on Manufactured Nanomaterials (WPMN) as a subsidiary body of the OECD Chemicals Committee. This programme concentrates on human health and environmental safety implications of manufactured nanomaterials (limited mainly to the chemicals sector), and aims to ensure that the approach to hazard, exposure and risk assessment is of a high, science-based, and internationally harmonised standard. This programme promotes international co-operation on the human health and environmental safety of manufactured nanomaterials, and involves the safety testing and risk assessment of manufactured nanomaterials.

The objective of this document, *Guidance Manual for the Testing of Manufactured Nanomaterials*, is to assist sponsors in the development of *Dossier Development Plans* (DDPs) which describes the testing programme for specified Manufactured Nanomaterials. It is intended to ensure that the information collected from this testing programme be reliable, accurate and consistent. It is important to note that this *Guidance Manual*, is a "living document" and as such, it is expected to be updated and amended in an iterative manner based upon knowledge accumulation, evolving communication and coordination needs as the testing programme and work on the DDPs progress. More information on the testing programme 'the Sponsorship Programme" is available in the Executive Summary and Chapter II of this document.

The WPMN finalised a first version of this document, which was published in 2009 with the agreement of the Joint Meeting. As a follow-up, the WPMN developed a *Data Sharing Template Format* for its inclusion in this guidance document as Annex III. In addition, another annex, Annex IV, has been included in this document which covers *Alternative Methods in the Sponsorship Programme*. Accordingly, this updated document replaces the publication number [ENV/JM/MONO(2009)20].

This document is published on the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

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THE WORKING PARTY ON MANUFACTURED NANOMATERIALS (WPMN)

The Working Party on Manufactured Nanomaterials was established in 2006 to help member countries efficiently and effectively address the safety challenges of nanomaterials. OECD has a wealth of experience in developing methods for the safety testing and assessment of chemical products.

The Working Party brings together more than 100 experts from governments and other stakeholders from: a) OECD Countries; b) non-member economies such as China, the Russian Federation, Singapore, South Africa, and Thailand; and c) observers and invited experts from UNITAR, FAO, UNEP, WHO, ISO, BIAC[1], TUAC[2], and environmental NGOs.

Although OECD member countries appreciate the many potential benefits from the use of nanomaterials, they wished to engage, at an early stage, in addressing the possible safety implications at the same time as research on new applications is being undertaken.

The Working Party is implementing its work through specific projects to further develop appropriate methods and strategies to help ensure human health and environmental safety:

- OECD Database on Manufactured Nanomaterials to Inform and Analyse EHS Research Activities;
- Safety Testing of a Representative Set of Manufactured Nanomaterials;
- Manufactured Nanomaterials and Test Guidelines;
- Co-operation on Voluntary Schemes and Regulatory Programmes;
- Co-operation on Risk Assessment;
- The Role of Alternative Methods in Nanotoxicology;
- Exposure Measurement and Exposure Mitigation; and
- Environmentally Sustainable Use of Nanotechnology.

Each project is being managed by a steering group, which comprises members of the WPMN, with support from the Secretariat. Each steering group implements its respective "operational plans", each with their specific objectives and timelines. The results of each project are then evaluated and endorsed by the entire WPMN.

More information about the work of the WPMN, as well as publications and updates on efforts of governments and other stakeholders to address safety issues of nanomaterials is available at http://www.oecd.org/env/nanosafety.

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1. The Business and Industry Advisory Committee to the OECD
2. Trade Union Advisory Committee to OECD

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EXECUTIVE SUMMARY

The WPMN project *Safety Testing of a Representative Set of Manufactured Nanomaterials* builds upon the concept that much valuable information on the safety of manufactured nanomaterials (MNs), as well as the methods to assess safety, can be derived by testing certain nanomaterials for human health and environmental safety related effects. This project led to a Sponsorship Programme for testing a set of manufactured nanomaterials using appropriate test methods, which would include OECD Test Guidelines or other internationally agreed methods.

As a first step, the WPMN agreed a list of fourteen representative manufactured nanomaterials and a list of endpoints including nanomaterial information/identification, physical-chemical properties and material characterisation, environmental fate, environmental toxicology, mammalian toxicology and material safety, which would be addressed for the hazard assessment of those nanomaterials. As a second stage, the WPMN launched the *OECD's Sponsorship Programme on the Testing on Manufactured Nanomaterials*, to generate information on the safety of the specific manufactured nanomaterials through testing for human health and environmental safety endpoints[3].

The "Sponsorship Programme" is a process to conduct, as appropriate, specific tests for manufactured nanomaterials. It involves OECD member countries, as well as some non-member economies and other stakeholders solely or in partnership with other entities, to pool expertise and to fund the safety testing of specific Manufactured Nanomaterials (MNs).

In order to assist sponsors in the development of *dossier development plans* (DDPs) which describes the testing programme for a specified MN, the WPMN agreed to develop this *Guidance Manual*. It is intended to ensure that the information collected from the Sponsorship Programme be reliable, accurate and consistent.

The *Guidance Manual* builds upon the work already achieved by the WPMN. The work of the WPMN project on the *Safety Testing of a Representative Set of Manufactured Nanomaterials* (SG3) was complemented by other WPMN projects. In particular, by the projects on: *Manufactured Nanomaterials and Test Guidelines* (SG4); and *The Role of Alternative Methods in Nano Toxicology* (SG7).

- *Project 4: Manufactured Nanomaterials and Test Guidelines*: This project addresses whether existing test guidelines (used for "traditional chemicals") can be successfully applied to manufactured nanomaterials. Thus, this project is reviewing existing test guidelines [especially the OECD Test Guidelines (TGs)] with view to establishing whether they are suitable for manufactured nanomaterials. Test Guidelines include: physical chemical properties; effects on biotic systems; degradation and accumulation; and health effects. In addition, a document, *Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials,* was published in 2009 and another document, *Preliminary Guidance Notes on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials,* has also been recently published in 2010, as an outcome of this project.
- *Project 7: The Role of Alternative Methods in Nanotoxicology*: This project is looking into alternative test methods (to reduce the use of animals in the hazard evaluation of nanomaterials) and will analyse how they might be used in an overall assessment plan for hazard testing of

[3] It is worth noticing that it is not expected that the all endpoints included in the list will be suitable to test all of the MNs. However, based on the practical results obtained from the first phase of the testing programme, it is expected that a clear indication will be provided on the appropriateness of the listed endpoints for those selected manufactured nanomaterials, as well as the need for further testing involving additional endpoints.

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manufactured nanomaterials. In addition, this project is developing text on testing strategies and *in vitro* methods for human health hazard identification to complement the Guidance Manual.

This Guidance Manual includes the appropriate protocols and methodologies. It also: i) describes the three modes of sponsorship involvement (lead-Sponsors, co-Sponsors and Contributors); ii) explains the distinction between a Phase 1 (exploratory) and a Phase 2 of the Sponsorship Programme; iii) provides considerations on how to develop a sufficient set of information to address endpoints; iv) outlines the WPMN review and oversight approaches, including a two-year review; and v) outlines those expected outputs from the testing programmes of each sponsored manufactured nanomaterial (MN).

This document was first developed by a Task Group on Guidance Manual for Sponsors, which was established under the projects *Safety Testing of a Representative Set of Manufactured Nanomaterials (SG3)* and *Manufactured Nanomaterials and Test Guidelines (SG4)*. Also, the project on *The Role of Alternative Methods in Nanotoxicology (SG7)* provided specific inputs regarding *in vitro* methods for human health hazard identification. More information is available on *Alternative Methods in the Sponsorship Programme* in Annex IV. Finally, the main text was prepared in an iterative manner with sponsors as the work progressed and was endorsed by the WPMN at its 5th meeting in March 2009. A *Data Sharing Template Format* was finalised at the 6th meeting in October 2009 and it was agreed to its inclusion to this document as an annex (Annex III).

It is important to note that this *Guidance Manual for the Testing of Manufactured Nanomaterials* will continue to be updated and amended in an iterative manner based upon the accumulation of knowledge, as well as evolving communication and coordination needs as the testing programme and the development of DDPs moves forward.

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CHAPTER I
INTRODUCTION TO THE GUIDANCE MANUAL

BACKGROUND

OECD's Working Party on Manufactured Nanomaterials (WPMN) established a project entitled "Safety Testing of a Representative Set of Manufactured Nanomaterials" (SG3) to identify and test a representative set of manufactured nanomaterials using appropriate test methods that would include OECD Test Guidelines or other internationally agreed methods.

The WPMN agreed that the project would move forward in two stages, the first stage being to develop and agree to a priority list of representative Manufactured Nanomaterials (MNs)[4] for inclusion in a set of reference nanomaterials for which development of data would support characterisation, measurement, toxicological and ecotoxicological testing, and risk assessment or safety evaluation of MNs. The second stage will develop a programme to create a better understanding of MNs that may be relevant for exposure and effects of nanomaterials by testing representative nanomaterials for human health and environmental effects, as well as physical-chemical properties and environmental fate for a specific set of endpoints. It was agreed that the dataset developed through this programme would be of an exploratory nature. This programme would also be science-based, open to all stakeholders and without pre-defined regulatory consequences for any datasets.

At the third meeting of the WPMN (November 2007), the list of representative MNs and list of endpoints were adopted and recommended to be declassified by the Chemicals Committee (see Annex I). At the same time, the WPMN agreed to a recommendation for launching a "sponsorship" testing programme[5] for certain MNs. Such a "Sponsorship Programme" establishes a process to conduct, as appropriate, specific tests for those endpoints on those MNs agreed by the WPMN, which will be described in Dossier Development Plans for each MN.

To aid Sponsors in the testing programme and to build upon the work already achieved by the WPMN projects on: i) the Safety Testing of a Representative Set of Manufactured Nanomaterials (SG3); and ii) on Manufactured Nanomaterials and Test Guidelines (SG4); it was agreed that a guidance manual for sponsors would be useful. Accordingly, in February 2008, a Task Group was established to develop the Guidance Manual. It was agreed that the Guidance Manual should be a document which enables the drafting of Dossier Development Plans and the Sponsorship Programme itself to be reliable, accurate and

[4] The phrase "representative manufactured nanomaterials" was intended to mean those manufactured nanomaterials (MNs) now or soon to enter into commerce. It was not intended to mean representative of class or category of manufactured nanomaterials.

[5] As referred to here a "sponsorship" involves individual entities, such as members of the OECD WPMN solely or in partnership with other entities, developing and carrying out information-development programmes (such as conducting data analyses or developing data using appropriated characterisation or toxicity tests) to address the endpoints as identified in this Guidance Manual for one or more representative MNs of Annex I. The "Sponsorship Programme" refers to the overall organisation of the OECD testing programme for MNs within which sponsors will conduct and report the results of their information development programmes.

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consistent. There was agreement that the appropriate protocols and methodologies should be included in the Guidance Manual.

OBJECTIVE

The objective of the Guidance Manual is to assist Sponsors in the development of comprehensive and consistent Dossier Development Plans (DDPs). A DDP is a core document describing the testing programme for a specified MN including the identification of all participants, selection of the test materials, and test designs to address[6] all endpoints. A DDP also communicates the state of understanding for each endpoint and the plans for completion of addressing each endpoint in consideration of that understanding. A draft dossier for a specified MN will be developed to present the outcomes and results of testing for each endpoint.

In addition to aiding those participating in the Sponsorship Programme, the Guidance Manual will also serve as an informational source for those seeking information on the goals and objectives of Phase 1 of the Sponsorship Programme and provide a basis for the WPMN to review DDPs.

ADDITIONAL INFORMATION

In this Guidance Manual, testing methods for certain endpoints are subject "to be determined (TBD)" see Section 2 under *Endpoints for Testing Nanomaterials*. This is because the WPMN recognises that there are no testing methods for the endpoints to recommend at the time of development of this document. The WPMN will keep abreast of developments in the area to update Sponsors. The WPMN also expects that the Sponsorship Programme itself will fill the gaps of undeveloped testing methods. At this time, the sponsorship programme is less a data development programme and more a methods development programme than the WPMN had originally anticipated.

A *Data Sharing Template Format* has been developed (See Annex III). It is intended to provide guidance on how to report results within the programme.

A text on *Alternative Methods* in the Sponsorship Programme is found at ANNEX IV. As with other sections of this document, this annex is a "living" text and will be updated with future revisions.

It is expected that the *Guidance Manual* will continue to be updated and amended, as needed, based upon knowledge accumulation, evolving communication and coordination needs.

[6] See "Phase 1" on page 17 for the definition of "address". Note that for some endpoints (for example, solubility) it is specified that the endpoint must be "completed". In such instances "completed" means that all Dossiers will be providing this endpoint information. To improve confidence in reported data round-robin testing or other approaches may be performed.

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CHAPTER II
THE SPONSORSHIP PROGRAMME

SECTION 1: SCOPE AND TERMS

The Sponsorship Programme is based upon the belief that valuable and relevant information on the safety of MNs and the methods used to assess safety can be derived by appropriately testing a representative set of MNs for identified human health and environmental safety endpoints. The WPMN has determined that the programme is intended to develop the data that will improve the understanding of MNs, and, if possible, to understand what information may be generalised across different MNs or classes of MN.

The Sponsorship Programme is being organised in two phases.

Phase 1

In Phase 1, the WPMN has invited WPMN participants (including all stakeholders) to volunteer to sponsor the testing of one or more of the MNs on the list of representative MN, shown in Annex I. Sponsors have been asked to prepare a Dossier Development Plan (DDP) for the testing of the MN. The DDP will be reviewed by the WPMN. Based upon the reviewed DDP, Sponsors would complete Phase 1 by addressing those endpoints in Annex I appropriate for the material. The scope of Phase 1 is to provide a dataset by addressing the endpoints listed in Annex I. This includes, where appropriate, the utilisation of existing relevant information, the generation of new information or the rational why the information is not needed. Also where specified in the Guidance Manual, "address" includes the term "completed" which provides that all dossiers will contain the identified endpoint information.

An MN-intrinsic property is a property of a MN that is unique to the nanoscale features of that material. An MN-intrinsic property generally would not be expressed in particles or other features of the material that are not in the nanoscale range. The term "MN-intrinsic" is not meant to define scope for this guidance because properties that are "MN-intrinsic" cannot be comprehensively defined *a priori*, so data development efforts cannot be limited to them. The development of data for the Sponsorship Programme will generally be directed toward properties of MNs for the listed endpoints. MN-intrinsic properties will become better understood through the development of these datasets.

In any case, where it is not feasible or not appropriate to develop test data for an endpoint, a rationale for not testing must be provided. Phase 1 is exploratory in nature as the testing methodology and strategy may need to be developed and might evolve during Phase 1 testing. With regards to test methodology, the Phase 1 testing should also serve to inform test guideline development within the work activities of the WPMN SG4. Further, as far as possible and appropriate, a full dataset shall be generated for each MN independent from decision logic based on risk management considerations. The sum of datasets generated by Phase 1 testing together with the methodology developed and experiences gained will help to understand properties specific to the nanoscale features of MNs and to identify data to be developed in Phase 2 testing.

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Phase 2

As informed through addressing endpoints in Phase 1, Sponsors would determine additional appropriate and feasible testing needed and develop the dossier plans as needed for Phase 2 testing. Note that it is not the intention that initiation of Phase 2 must be delayed until all of Phase 1 is complete. As Phase 1 and 2 are distinct commitments, the Sponsors for Phase 2 may or may not be the same as in Phase 1. The scope of Phase 2 is to address additional endpoints that are necessary to gain understanding of the hazard potential of the respective sponsored MN. The combined data provided by Phase 1 and Phase 2 testing will allow, but not necessarily be entirely sufficient for, application to risk assessment paradigms as considered for specific sponsored MN applications (given that adequate exposure data are available). Phase 2 would also be where such aspects as life cycle of MNs and evaluation of by-products of the use of nanomaterials could be considered in greater depth and specificity than may be possible in Phase 1. Thus, Phase 2 testing may be guided by risk related decision logic.

This Guidance Manual is intended to cover only Phase 1 of the Sponsorship Programme, but will necessarily include reference to some items that may be considered in Phase 2 testing.

The "exploratory nature" of Phase 1 of the Sponsorship Programme, as described by the WPMN, determines the intention and the nature of testing but not the scope with regard to completion or approximate time and effort involved. Furthermore, it is recognised that some test methods will need to be developed in a research framework in order to address endpoints; however, it is not the intention of WPMN that the Sponsorship Programme becomes an open ended research programme. Therefore, as a practical approach to diminish this potential for open-ended research, it is proposed that a 2-year time frame (roughly two months prior to the 8th meeting of the WPMN in early 2011) be set at which time an interim assessment step of Phase 1 will be undertaken. A report regarding status of the programme would be presented to the WPMN at that time. This Guidance Manual and the individual DDPs will form the basis for organisation of the interim status assessment.

At the conclusion of this two-year period of time, the WPMN and Sponsors would consider the following:

1. The status, need for, and coordination of further test methods development;
2. Assessing of the state of understanding of MN-intrinsic properties;
3. Whether and what to begin moving to the Phase 2 testing and Programme
4. What is needed and what the timeline is for completing Phase 1.

Key Terms

The Sponsorship Programme has three levels of participation available. The three levels of participation are lead Sponsors, co-Sponsors and Contributors. The responsibilities for each level of participant are as follows:

- **A Lead Sponsor** assumes responsibility for conducting **<u>or co-ordinating</u>** all of the testing determined to be appropriate and feasible to address the endpoints of Phase 1 for a listed MN. In some cases, considering the degree of participation committed toward addressing endpoints, "joint lead" arrangements may be developed where appropriate. In this Guidance Manual, the Lead Sponsor and joint lead arrangements are generally referred to as the "Sponsor."
- **A Co-Sponsor** conducts some of the testing determined to be appropriate and feasible to address the endpoints of Phase 1 for a specific listed MN.
- A **Contributor** provides test data, reference or testing materials or other relevant information to the lead and co-Sponsors.

Note that whereas the Lead Sponsor(s) have the ultimate responsibility for development of the testing dossier, being a Co-Sponsor also allows participants to have an active role in preparing the DDP. Contributors, by request of Lead Sponsors, could participate in developing DDPs. It is expected that the Lead Sponsors for each of the selected MNs will communicate and share information among them as appropriate. An overall Communication Strategy should be developed as part of the DDP.

MNs referred to in this document can be conceptually categorised into:

- A MN as a general term to refer to all MNs.
- A "representative MN" refers to a MN that is on the list in Annex I and is now or soon to be in commerce.
- A specific MN variation or "sponsored MN" within the variations possible for "representative MNs" in Annex I, such as a single-walled carbon nanotube made by a specific process and with defined physical characteristics. A specific MN variation is selected by Sponsors as the material for which they will address the endpoints in Annex I during Phase 1.

The following terms should be used for description of study materials in DDPs,

- Principal nanomaterial – refers to sponsored MN(s) tested on for all endpoints
- Alternate nanomaterial – refers to sponsored MN(s) that are tested on some, but not all endpoints
- Control – refers to comparison non-nanomaterials, such as coarse materials, ionic material (e.g. ionic silver when compared to nanoscale silver particles), or impurities (such as nickel catalyst for single-walled carbon nanotubes).
- The elements identified in Annex II must be **Completed** for each principal nanomaterial, alternate nanomaterials and control material.

Box 1: Example of the use of terminology to refer to studied materials in DDPs

Three MNs are proposed for testing within the MWCNT DDP as presented at the Busan Workshop in November 2008. The three MNs are:

1. Nikkiso MWCNT (Manufacturer: Nikkiso Co., Ltd.)
2. Graphistrength C100 (Manufacturer: Arkema France)
3. Mitsui MWCNT (Manufacturer: Nano Carbon Technologies Co., Ltd.)

The MWCNT DDP proposes to address all endpoints in Annex I of this guidance for the first MN, and so this MN should be termed a "principal" MN in the DDP and in the reporting template (see page 47). Additional "principal" MNs could be included in DDPs in general if all endpoints were also addressed for those additional MNs.

The MWCNT DDP further proposes to conduct tests for the second and third MNs listed here, but does not propose to test all endpoints. The information developed from the testing of the second and third MNs would add to the information used to address some endpoints, but it would not be sufficient on its own to address those endpoints (in the absence of information from the "principal" MN). The second and third MNs should be termed "alternate" MNs in the DDP and in the reporting template (see page 47).

For DDPs that propose testing of a coarse variation of the material in terms of size of particles (for example, the metal oxides with particle sizes ranging in the micron scale), the coarse material should be referred to as a "control" material in the DDP. If DDPs were to propose testing a predominant contaminant of the MNs, then that test material would also be called a "control" material.

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Definition of terms related to "Addressing" an endpoint:

All endpoints must be 'addressed'. Possible ways to 'address' an endpoint are: 'not relevant'; 'not measurable'; 'read-across'; and 'data provided'. Data must be provided for endpoints indicated as 'this element must be completed' (i.e. 'completed' = 'data on principal material provided'). The term "Addressed" means that the endpoint can be satisfied by providing information that explains why the endpoint is:

- Not Relevant – For example, aerobic biodegradability is Not Relevant for metal oxides as they are already completely oxidised.
- Not Measurable – For example, the Space Group of an amorphous material such as amorphous silica is Not Measurable.
- Read-Across Information – Read-Across Information is the transfer of property or toxicological information of one nanomaterial to another with a similar structure or composition. For example, data for the porosity for an MN may be able to be Read-Across for a surface treated version of the same material.
- Data Provided – Data is provided that specifically applies to the principal MN.

The term "Completed" means that the endpoint can only be satisfied by Data Provided. The endpoints that must be **Completed** are those included in Annex II.

There is a preference for ***exploration of sponsored MN properties*** in Phase 1 testing rather than developing specific data for ***risk management purposes***. It is not the intention that addressing the endpoints in Phase 1 will support development of a risk estimate. Rather, specific properties relevant to characterising risk are becoming clearer through exploratory research. Therefore, further research of an exploratory nature is needed in order to inform the development of test methodologies and choices for which sponsored MN variations would potentially be carried forward to Phase 2 testing so that the resulting data developed in Phase 2 are relevant to risk management purposes.

The information used to address an endpoint can come from a variety of sources, and it is the intention of WPMN that this guidance should be flexible enough to allow incorporation of existing information and ongoing test programmes where feasible. Whether developed by Sponsors or contributed from other sources, ensuring that data are generated in accordance with Good Laboratory Practices[7], where applicable, will help ensure that information used to address endpoints is reproducible and of high quality. Also with respect to reproducibility, for some endpoints the utility of round-robin testing may be considered.

Information used to address endpoints can include the use of tests or test batteries to develop new data that employ OECD test guidelines such as those for chemicals, existing non-OECD tests (for example, assays promulgated through standard development organisations (SDOs) or scientific research development programmes, or tests that have been newly developed for the purpose of this Sponsorship Programme. In all cases the use of tests should be communicated between Sponsors so that similar methods can be used and similar reporting elements can be assured (through adoption of similar methods and instrumentation wherever feasible) in the resulting data sets. This will facilitate consistency and harmonisation in methods use and data generation across sponsorship programmes for different MNs and cross-MN comparisons so that the most can be gained from structure-activity relationship modelling and other extrapolation and integration activities that may arise from the test programme.

[7] http://www.oecd.org/env/glp

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Information used to address endpoints can also include use of existing published data. However, use of such data should be restricted to those cases where data are deemed relevant and of appropriate quality including but not limited to consideration of:

- Thorough characterisation of the tested material as available in the studies cited or as provided in subsequent reports;
- Peer-reviewed methodologies are used where available and appropriate;
- Standardised procedures/assays/models are used where available and appropriate;
- Whether the material tested is the same as the sponsored MN or aids in addressing an endpoint by improving understanding of the sponsored MN.

Consistent reporting of the data used to address endpoints will facilitate comparisons within and between Sponsor programs so that the most can be gained from data integration and extrapolation efforts that may arise from the test programme. Information from published studies should also be robust[8] with respect to exploration of properties[9].

Information used to address endpoints can also include weight of evidence and expert opinion to evaluate data sets. For example, comparison of test results for a sponsored MN to test results for other forms of the material (non-nanoscale forms) will be informative to the understanding of the "MN-intrinsic" properties of the sponsored MN. Such comparisons to non-nanoscale forms will also reduce the likelihood that false positive results with respect to the nanoscale form are inferred from studies of the sponsored MN. In other words, if a result is seen for the nanoscale and non-nanoscale forms, then the result is not necessarily a function of the nanoscale elements of the sponsored MN. Such comparisons of information from nanoscale and non-nanoscale forms of a material should be made at the discretion of the Sponsors in addressing the goal of improving the understanding of the sponsored MN for particular endpoints in Annex I. The information should be made available in a robust study report.

Phase 1 commitment by Sponsors means addressing the entire list of endpoints of Annex I for a sponsored MN. It is anticipated that interim evaluation phases would be undertaken by Sponsors in collaboration with other Sponsors and OECD. In those interim evaluations, a Sponsor would identify gaps in information needed to address the endpoints and data and methods development plans to address the gaps, if any.

It is also anticipated that, during initial phases of addressing endpoints, Sponsors will need to evaluate what methods are appropriate to explore the properties of the sponsored MN for a given endpoint. This evaluation may result in plans to perform "round robin" testing; identification of needs to develop new test methods and instrumentation; and the description and initiation of programmes to develop methods needed to address endpoints.

Methods development within the Sponsorship Programme is likely to be an ongoing and iterative process, involving research and development, and new understanding. Furthermore, methods for one sponsored MN will be informed by those for other sponsored MNs. Therefore Sponsors for different sponsored MNs are encouraged to communicate test method development plans and needs with other Sponsors and other relevant parties (national testing programmes, alternate methods validation entities, standard development organisations) so that, where feasible, efficiencies of scale and consistency across

8. The term "robust" means that study results can be readily repeated using the information provided and so are not likely to be subject to uncontrolled, unmeasured, and unreported variables. Given that there are reports of difficulty in repeating studies so that the same results are achieved, one means to assure that results are robust would be to conduct "round robin" repeating of a given test.

9. Manual for Investigation of HPV chemicals should be referred to here to aid in transparency of data utility decisions.

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sponsored MNs can be achieved. Workshops on endpoints or sets of similar endpoints should be initiated to accomplish the intention to promote consistency.

The methodology used for addressing a specific endpoint will be determined at the discretion of the Sponsor. Full information on methodology should be supplied to the Secretariat. In general, OECD test guidelines, as reviewed by the WPMN are preferred[10]. Methods developed by Standards Development Organisations (SDOs) should also be considered if OECD methods are either inadequate or not available.[11] Methods from non-SDOs may be appropriate in some instances. In some cases, adaptation of existing methods will be necessary for their use with nanomaterials. However, given the exploratory nature of the testing programme it is anticipated that new methods will be developed in some cases for which similar or adaptable OECD or other standardised methods are not available. It will be particularly important to ensure that study designs, data availability, and reports provide sufficient information so that the studies can be independently repeated.

Alternative Methods in the Sponsorship Programme

Alternative methods are an integral part of the WPMN's work in the Sponsorship Programme. This will be particularly relevant as the work of the WPMN project *The Role of Alternative Methods in Nanotoxicology* (SG7) evolves. It is foreseen that this section is developed in parallel with OECD's Working Group of the National Coordinators of the Test Guidelines Programme (WNT)[12] activities on the validation of the test guidelines and forms a part of the development of an overall strategy for reducing the use of animals in the hazard evaluation of nanomaterials.

An alternative method is a test that: (i) reduces the number of animals required; (ii) refines procedures to lessen or eliminate pain or distress to animals, or enhances animal well-being; (iii) or replaces animals with non-animal systems or with less sentient species. This refers directly to the 3Rs principle of refinement, reduction and replacement, which should be respected in the sponsorship programme. Modern toxicology makes use of integrated testing strategies (ITS). This includes application of a weight-of-evidence (WoE) approach for certain toxicological and ecotoxicological endpoints or information requirements. This WoE takes into account all available sources of information and types or formats of data. ANNEX IV, *Alternative Methods* in the Sponsorship Programme is an initial text, which will be updated with future revisions of the document.

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10. The WPMN conducted a "Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials" and the document is available for the use of Sponsors.

11. Currently the following standards development organisations are active: ISO/TC229 – Nanotechnologies, ASTM E56 Nanotechnologies, CEN/TC352, There are several programmes which focuses on the test methodology development as follows: NanoInteract, NanoDerm, and the NCI Nanotechnology Characterisation Laboratory

12. http://www.oecd.org/env/testguidelines

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SECTION 2: DESCRIPTION OF DOSSIER DEVELOPMENT PLAN

This section of the Guidance Manual describes the content of the DDPs. The goal of this section is to assist Sponsors in preparing the DDP, and to ensure that DDPs are comprehensive, consistent and comparable among Sponsor groups. In the DDP, Sponsors will describe all aspects of the Phase 1 exploratory research including identification of sponsorship participants and their responsibilities, selection of test materials, specific endpoints to be addressed, methods and approaches for addressing endpoints, justifications for not using OECD Test Guidelines, experimental design, and an approximation of timelines for reporting results. It is requested that Sponsors be concise and as brief as possible in writing the DDP.

The *Introduction* section of the DDP will provide a thorough, but brief review of the existing literature pertinent to addressing the endpoints in Annex I for the selected test nanomaterials. This section should be viewed as a key component of the DDP and the eventual dossier, as it provides a knowledge base that will be used by all OECD nanomaterial Sponsors, reviewers of the DDP within WPMN, and steering groups and task groups within the WPMN.

The organisation of this guidance document follows the intended section organisation of the DDPs that would be developed based on it. Critical decision elements are identified and described within each section and it expected that Sponsors will explain and justify the rationale used for making each decision. In a manner consistent with guidance in this document on the meaning of the term “address”, Sponsors are expected to identify and justify the use of existing data to address endpoints, as well as identify why existing data is considered inadequate and additional testing required. Decision elements in this case include, in part, applicability to understanding the properties of the sponsored MN, reliability, and consistency across studies for the sponsored MN endpoint and across studies used for other sponsored MNs in the OECD test programme of these existing data.

OUTLINE OF DOSSIER DEVELOPMENT PLANS

I. Introduction

The introduction should include a thorough but concise review of information available for the specific nanomaterial sponsored. The purpose of the *Introduction* section is to provide those reviewing DDPs with background information for the review process, to demonstrate a thorough understanding of existing information concerning the nanomaterials to be tested, and to add information to the WPMN database on nanomaterials. This overview will also briefly describe in an overall sense the state of available information for the selected nanomaterial relative to the list of sponsorship endpoints. Particular attention will be given to identifying where adequate endpoint information exists as well as where and why existing information is considered inadequate for the Sponsorship Programme. Detailed discussion of whether and how an endpoint is addressed would occur at the point in the DDP where research plans and existing data for endpoints are presented in turn.

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II. Identification of Participants

In this section, all sponsorship participants will be identified and their affiliations, contact information provided. The sponsorship lead or co-leads will be identified. The logistics of Sponsor interaction will be discussed, and will include:

- Which technical/scientific competence and laboratory management quality criteria or indicators will be used when searching/selecting/evaluating candidate participants for the tests;
- How tests will be coordinated and who will run and be responsible for them;
- When and how Sponsor meetings will be held;
- How information will be exchanged among sponsorship participants;
- How data and other information will be sent to the WPMN clearinghouse; and
- Who will be responsible for final reports to the WPMN.

III. Communication Strategy

Sponsors will outline their communication plan in accordance with the work programme, timeline, deliverables and milestones of each Sponsor's and Contributor's research plan; alignment of the individual research plan elements within a single agreed communication plan will form the *Communication Strategy* of a given representative material. The Communication Strategy should describe the following aspects:

- Communication plan between Lead Sponsor(s) and the OECD WPMN (including format, timeline, deliverables, and milestones)
- Communication plan between Co-Lead Sponsors (including form of agreements, agreement processes, final responsibilities of communication of consolidated results to the OECD WPMN)
- Communication plan between Lead Sponsors, Co-Sponsors, and Contributors, (including format, timeline, deliverables, and milestones)

IV. Material Selection

In this section, Sponsors will describe all aspects of the material selection process in consideration of the guidance in this document. While much of the background information on the selected nanomaterial will be discussed in the *Introduction* section, Sponsors will thoroughly describe their rationale for decisions regarding material selection. These decisions will be described in subsections including:

Rationale for material selection

Sponsors will explain their rationale for selection of specific materials. This will include discussion of the bases of decisions on such issues as selection of a specific nanomaterial production method, level of purity, level of functionalisation, etc. These decisions might be based on levels of hazard indicated in the scientific literature, potential for exposure (i.e., production volume or specific product use), how representative a specific material is to nanomaterials in general, and other information which should be thoroughly discussed.

A DDP should at a minimum address all endpoints for one nanomaterial (the "sponsored MN") but may include plans to develop information using any number of other nanomaterials or other materials. The sponsored MN samples or batches used in the tests used to address endpoints should be as close to being "identical" as can be reasonably ascertained using relevant characterisation tests, including those in

Annex I under "Nanomaterial Information/Identification and Physical-chemical Properties and Material Characterisation"[13]. To aid in assuring the identical nature of the sponsored MN, the material used in different tests should be obtained preferably in a single lot, and stored and manipulated in comparable, if not identical procedures. It should be noted, however, that Sponsors might choose to test and develop DDPs for two (or more) similar sponsored MNs, for example, single-walled carbon nanotubes produced by different methods.

It is anticipated that some Sponsors will choose to test other MNs in addition to the sponsored MN when exploring the effect of variation in properties on an endpoint. For example, the effect of changes in size distribution in the nanoscale range might be explored for zinc oxide by testing more than one variety of nanoscale zinc oxide in an acute toxicity assay. In doing so, sponsors may wish to consider exposure scenarios throughout a life cycle of a MN.

Identification, source, logistics of distribution

Sponsors will identify the source of test nanomaterials, including all known aspects of material production, the manufacturer, facility location, lot number, and any other pertinent information as noted in Annex I "Nanomaterial Information/Identification". The goal of this section is to aid in the comparison of other tests performed on the material, acknowledging the probability that materials can differ among production runs or lots. The logistics of material distribution among Sponsor laboratories will be described, as will approaches to all aspects of handling and storage that might introduce material variation into the Sponsors activities.

The following points should be considered and reasons for decisions documented as a minimum set of consideration and are not exclusive; other considerations the Sponsors want to introduce are welcome:

a) The sponsored MN selected for the work programme is part of the list of representative MN identified by the WPMN and contained in Annex I or its revisions.

b) It is "representative" in terms of production volume (present or expected), and/or in terms of potential environmental or human exposure.

c) The programme proposed takes into account the existing available background information and knowledge concerning the product in nanoscale and non-nanoscale forms. In particular, it identifies the tests already performed and knowledge gaps to be addressed. In the case of non-nanoscale forms, information on production volumes issues may affect choices of sponsored MNs that are most likely to provide data relevant to MNs likely to be used in commerce and to which exposures may occur. Known toxicities of non-nanoscale forms, based for example on the chemistry or physical properties of those materials, may also influence choices of sponsored MNs.

d) The sponsored MN can be made available to the Sponsors, co-Sponsors and Contributors, based on agreement with suppliers in a manner that is satisfactory to the understanding of the materials properties for the OECD test programme (for example, in consideration of confidential business information with regard to production of the material and reporting of its properties). The involvement of the supplier in the Sponsorship Programme is recommended in order to benefit from the supplier's established knowledge concerning the product and its properties, and their possible evolution. In particular, benefits hereunder will be envisaged as advantages of such suppliers involvement:

[13] Also note ISO Guide 35:2006 - Reference materials -- General and statistical principles for certification, or Linsinger et al., Accred. Qual. Assur., 6, 20-25 (2001)

- The sponsored MN is consistently and readily available from cost and timeframe point of view;
- A "master batch" of a sufficient quantity for Phase 1 can be made available to ensure a constant supply of the same sponsored MN in consistent quality during all the testing phase. By "consistent quality", we intend homogeneity in properties and consistent sampling procedure; the homogeneity of the material should be assessed in dedicated homogeneity tests for a number of critical properties;
- The supplier is able to provide, with each batch of the sponsored MN delivered, a basic set of properties to be checked immediately prior to the delivery, to ensure a consistent supply with time;
- The sponsored MN can also easily be produced in smaller batches for specific tests of variants such as coatings, labelling, high purity material or any change in physical-chemical properties needed to understand the mechanisms of toxicity;
- The storage conditions are such as not to lead to significant changes in the sponsored MN properties with time. The stability of the material, both under conditions of transport and distribution (short-term) as under conditions of storage (long term) should be assessed in dedicated stability tests for critical properties, including those in Annex I under "Physical-chemical Properties and Material Characterisation"[14]. Traceability and history of the sponsored MN are ensured;
- The conditions of production take into account potential biological contamination that could alter the test results. The eventual decontamination processes are documented and can be implemented without changing the basic properties of the batches, lots, or aliquots of the sponsored MN; and
- Production and characterisation information for the sponsored MN can be adequately determined, in consideration of intellectual property and confidential business information concerns.

e) The possibility of testing variations in the properties of the sponsored MN (for example in "shallow" testing of particular endpoints using a representative MN that is different from the sponsored MN) is taken into account to foresee the potential variability of the product on the market:

- Close variations of the sponsored MN are available from other producers and/or can be produced by other processes (e.g., wet/dry processes, mechanical/chemical, etc…).
- The potential variants of the sponsored MN (for example, shape, crystal phase, composition) are documented and can be produced in a controlled and reproducible way.

[14] Also note ISO Guide 35:2006 - Reference materials -- General and statistical principles for certification, or Linsinger et al., Accred. Qual. Assur., 6, 20-25 (2001).

f) Standard operating procedures (SOPs) for sample preparation and testing have to be agreed upon between the Lead Sponsors and Co-Sponsors and the descriptions of the procedures should also be made available to the Contributors for their use. Contributors can furthermore participate in the agreement of SOP in terms of contributions they provide.

- Each SOP for sample preparation should result in minimal product transformation;
- The different surfactants/solvents and administration or dosing media are tested and have to be agreed upon between the Sponsors and Co-Sponsors (when relevant);
- Positive and negative controls are agreed between Sponsors and Co-Sponsors; and
- Exchange of information about the sample preparation and tests implementation protocols between Sponsors, Co-Sponsors, and contributors for different sponsored MNs is documented and transparent.

V. Discussion of Status

In this section, Sponsors will identify the state of progress made in **addressing each endpoint**. This will include, particularly at early stages of DDP drafting, a description of the review process for existing data and its incorporation in the overall analysis. While background information on the selected nanomaterial endpoints will be discussed in the *Introduction* section, Sponsors will describe their rationale for making several decisions regarding whether and how an endpoint has been addressed in this section.

Sponsors will also outline their approaches for further efforts needed, if any, to address each endpoint. This component can be in the last section of the "status" discussion for each endpoint and would be comprised of a brief description of the methods and approaches to be used to address it. Specific experimental-design aspects will be discussed in depth in a later section.

VI. Test Designs

In this section, Sponsors will provide specific description of endpoint tests and experimental designs. This section should also include presentation of timelines and milestones for completion of the testing program for the sponsored MN with respect to addressing each endpoint and reporting the data. Testing strategies used in this programme should aim at using minimum animal numbers for maximum information outcome with respect to specific endpoints. This would include combination of studies for several endpoints. Testing strategies used in the Sponsorship Programme should primarily focus on the endpoints identified as agreed by the WPMN and make best use of existing agreed test methods (OECD Test Guidelines, ISO standards or equivalent). Sponsors are asked to describe how they consider the use of alternative methods. In addition, where possible, the peculiar characteristics (nanoscale) of MN and their resulting possible capacity to reach and react directly at the cellular level should be taken into account. The WPMN believes, based on the nascent stage of nanomaterials research, it is in the best interest of the Sponsorship Programme to avoid being prescriptive in defining methods or approaches to addressing sponsorship endpoints. It is expected that Sponsors will thoroughly describe their rationales for selecting their experimental approaches, including efforts to seek efficiencies in design and data outputs through communication with OECD and Sponsors of other sponsored MNs that are also designing approaches. Such communication may occur through the OECD Secretariat or other mechanisms such as periodical technological meetings. It is recognised that in many cases these approaches will involve iterative processes where the results of preliminary testing guide the development of more definitive testing. This iteration would also benefit from interaction with other Sponsors in development of decision alternatives and choices for further action. The key elements of these decision processes should be described for each endpoint or endpoint category.

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Sample Preparation and Dosimetry

Sponsor(s) should determine, as part of the implementation of the DDP, protocols for how test materials will be handled to ensure that materials remain consistent across and within tests, including attention to specification of properties and hygiene of media used to store or administer the sponsored MNs.

Preparation of test samples and dose administration are critical considerations for the tests referred to or described in this guidance. For some nanomaterials, sample preparation and dosimetry may present complications not encountered with non nano-scale chemicals. For example, consideration should be given to the potential utility of dose metrics other than mass. The WPMN have identified questions regarding dosimetry, including what new measurement techniques will be needed to understand internal doses, and how to prepare and administer dosing material for *in vivo*/ *in vitro* studies for toxicity as well as for ecotoxicity, and fate and behaviour in the environment.

In general, Sponsors should be mindful of the potential for changes in the test material due to sample preparation and/or the nature of the test methods that may affect outcomes. Varieties of methods for creating suspensions of various nanomaterials have been documented in the literature but provide a starting point for subsequent or future standardisation or subsequent agreed guidance for standardisation or guidance on media preparation. This aspect of the Sponsorship Programme is expected to be highly experimental and exploratory. In the *Test Design* subsection, Sponsors should describe their approaches to producing, characterising, and quantifying nanomaterial suspensions used in exposures. Critical decisions to be addressed include suspension technique (e.g., sonication, stirring, use of solvents or emulsifiers, etc.), methods used to monitor nanomaterial properties within suspensions, and the frequency at which monitoring will occur. It is suggested that Sponsors familiarise themselves with applicable OECD Test Guidelines and the "Difficult Substances" document.[15] While these documents have been found to be deficient in describing methods for nanomaterials testing, they do describe critical goals for exposure stability (e.g., change in concentrations no greater than +/- 20 % of targets over the duration of exposure).

The Dossier should explain the reasons for the dose selection and, where appropriate, the rationale for vehicle selection. Methods for nanomaterial characterisation prior to and after use should be described in the Dossier, along with methods to characterise the nanomaterial in biological matrices. The Dossier should address the use of tissues (e.g., blood, liver) to conduct target organ dose metrics and possible metabolism or biological transformation of the sponsored MN. In order to assist sponsors, the WPMN has prepared *Preliminary Guidance Notes on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials*[16].

Furthermore, as many tests will be based on existing OECD Test Guidelines, for consistency in review, it will be useful to refer to those guidelines and describe modifications that have been used.

Identification of Critical Elements

In the test design sections for each endpoint, Sponsors should also identify aspects such as species and life stages to be tested, the source of organisms, all aspects of organism husbandry, general test

[15.] ENV/JM/MONO(2000)6 OECD Series on Testing and Assessment. Number 23. Guidance Document on Aquatic Mixture Testing of Difficult Substances and Mixtures

[16] Preliminary Guidance Notes on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials [ENV/JM/MONO(2010)25] is available at the OECD public website http://www.oecd.org/document/53/0,3343,en_2649_37015404_37760309_1_1_1_1,00.html

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procedures, endpoints that will be observed and the frequency of their observation. Sponsors should discuss these topics relative to the Sponsorship Programme list of endpoints as well as the unique nature of nanomaterials.

Considering possible changes in physical chemical properties during testing

The physical-chemical properties of an MN may change during the testing for the endpoint. This should be considered and, if appropriate, monitored during the test. The physical-chemical characterisations listed in Annex II shall be considered.

ENDPOINTS FOR TESTING NANOMATERIALS

The following subsection lists information intended to guide Sponsors in the selection of test methods and reporting elements that should be considered when addressing the endpoints for Phase 1 testing. Following the guidance should ensure consistency between the various tests to be carried out on specific sponsored MNs and the datasets developed. It should also lead to the timely development of a Dossier on a nanomaterial describing basic characterisation, environmental transport and fate, ecotoxicity and mammalian toxicity information. Sponsors should notice that some of those possible methods described hereunder may not fit for tests of nanomaterials and need some modifications when they conduct testing.

Nanomaterial Information/Identification

The following elements must be Completed.

Nanomaterial name

All known names by which the material may be accurately or commonly described must be provided. Examples include CAS Name, International Union of Pure and Applied Chemistry (IUPAC) Name, trivial name and common trade names if widely used.

CAS Number

Provide CAS number, if available.

Structural formula/molecular structure

An empirical formula and molecular structure must be provided. Each of these should be as precise as possible recognising that some materials may have some variable elements.

Composition of nanomaterial being tested (including degree of purity, known impurities or additives)

This is a critical element and must be provided since there may be nanomaterials that are described by the same name, but have critical differences. The purity should be expressed as the percentage of the intended nanomaterial present (e.g., 95% pure). The balance should also be described as completely as possible using percentages (e.g., 2% graphite, 1% MWCNT, 1% SWCNT, 0.5% Fe catalyst). See Annex II for additional information on the definition of **Composition**.

If additives are present in addition to the intended nanomaterial, they must be identified and the concentration provided. For example, if a nanomaterial is provided as a slurry or suspension, the concentration of the nanomaterial, the liquid and any additives such as surfactants must each be identified along with its concentration of the overall composition. Authors must consider if any treatments or

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additives change the character of the intended nanomaterial such that the results of the tests listed below may give misleading results.

Basic morphology

A high level description of the morphological nature of the intended nanomaterial must be provided. For example, is the material amorphous or crystalline? Are the particles spherical, rods, plates? A detailed description of this element may be provided in the Characterisation section below. See Annex II for additional information on the definition of **Shape**.

Description of surface chemistry (e.g., coating or modification)

Similar to the Composition element described above, if a nanomaterial has a functionalised surface, such as a treated, hydrophobic silica, the treating agent must be identified. Authors must consider if any treatments or additives change the character of the intended nanomaterial such that the results of the tests listed below may give misleading results. Unintentional functional groups on the surface such as those induced by purification processes must also be identified if feasible. See Annex II for additional information on the definition of **Surface Chemistry**.

Major commercial uses

Dossiers are initially intended to be developed for nanomaterials that are in commerce or soon to enter commerce. It can be expected that the commercial uses are known and should be described as completely as possible. Examples of uses for nanoscale materials include reinforcing agents such as in tennis racquets, anti-soiling agents such as on glass, antimicrobial agents, etc. Some nanomaterials may have more than one use. Optional information that authors could consider providing would be the uses of the non-nanoscale form of the material. In some cases a nanomaterial may be intended to replace a non-nanoscale material and the contrast in the uses may be informative.

Known catalytic activity

Known catalytic activity should be described.

Method of production (e.g., precipitation, gas phase)

The method of production must be described, particularly with respect to production methods that may affect key properties of the MN. For example, there are a variety of methods used to make carbon nanotubes including some that use catalysts. With any of the methods, whether or not a catalyst is used, there may be differences in the levels of impurities, tube length, number of walls for MWCNT, and so on. Another example is titanium dioxide where the method of manufacture may define whether the particles have an anatase, rutile or other form.

Physical-chemical Properties and Material Characterisation

Agglomeration/aggregation

This element must be **addressed**.

This property primarily addresses nanomaterial-nanomaterial interactions. Nanomaterials whether functionalised or not can interact with themselves by way of physical or chemical interactions. The presence of natural organic matter such as humic and fulvic acids play a role in determining this interaction and other charged polymers such as polysaccharides can be characterised for this purpose in

terms of their stickiness coefficients. The concept of agglomeration has great relevance for determining the fate and transport of nanomaterials because agglomeration rates can determine where nanomaterials segregate (i.e., water column to sediments) or else their transport can be effected, e.g., when the agglomeration remains in a dispersion.

The relevance of agglomeration/aggregation in alternate media such as solid phase or non-aqueous media also requires characterisation in order to determine the fate and transport properties of nanomaterials. For more information on media considerations, see Annex II.

The measurands of interest, beginning with a pre-determined unit of particles, are:

- The effective mean particle size in a given medium and its evolvement over time (including the standard deviation); and/or;
- qualitative assessment of state of aggregation and estimation of the primary particle size by TEM pictures
- Indirect confirmation of.the estimated primary particle size by BET measurements, for materials with low/no internal porosity as is typical for pyrogenic oxides. Porosity data should therefore be taken into account.

- Possible method(s):
  - Mean primary particle[17] size from powder TEM (for solids)
  - Mean particle size from PCS/DLS (for liquid dispersions)
  - Mean primary particle size calculated from BET surface area (for solids)
  - Mean particle size determined by SAXS
  - Mean particle size from SMPS (aerosol)

If none of the above are used, please specify the method used.

For some determinations it may be important to perform the evaluation after a set period of time in a standardised water or biological composition.

Water Solubility/ Dispersibility

This element must be **completed**. See Annex II for additional information on the term **Solubility**.

Water Solubility/Dispersibility refers to the mass proportion of a given sample of nanomaterial which is held in water solution or as a colloidal suspension in water as a function of time or where the sample of nanomaterial loses its particulate character as it changes from a particle form to a molecular form. It must be recognised that Solubility and Dispersibility are not identical though the distinction can be difficult to recognise with MN. The water conditions to which the MN is exposed must also be considered

[17.] Primary particle: Fused building blocks making up an aggregate. Primary particles as such are not freely available or accessible.

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as both solubility and dispersibility may be affected by pH, ionic strength, etc. For more information on media considerations, see Annex II.

The measurand of interest is, beginning with a pre-determined unit of particles in a standardised solution and temperature, to measure the mass proportion of nanomaterials which are held in solution, and:

i) whether this mass diminishes after a set period of time, or;

ii) Determine the amount of time required for mass to diminish by X%.

- Possible method(s): OECD TG105*Water Solubility*

Crystalline phase

This element must be **completed**. See Annex II for additional information regarding Crystallinity included within the definition of **Composition**.

Crystalline phase refers to the specific space group for a given crystal structure. In certain cases, it is possible to have multiple crystalline phases, such as with silica and titania. This will render definition difficult in some cases. The measurand of interest is fraction of the different crystalline forms present. For amorphous MN the lack of Crystallinity should be reported to satisfy this element.

- Possible method(s): X-ray diffraction, electron diffraction.

Dustiness

This element must be **addressed**.

'Dustiness is defined as the propensity of a material to generate airborne dust during its handling, and provides a basis for estimating the potential health risk due to inhalation exposure'[18].

The measurand of interest is the degree to which a given nanomaterial can remain in the air column before settling. This would require investigation and characterisation of interactions of nanomaterials with other common airborne particulate matter.

- Possible method(s): EN 15051:2006, DIN 33897-2, Vortex shaker method[19]

Crystallite size

This element must be **addressed**.

A crystallite is a part of a larger piece of material that has the same crystal structure and orientation. Typically a crystalline material consists of many crystallites packed together. If not, it is a "single crystal" or "monocrystal". This parameter only applies to nanomaterials with a defined crystalline structure (see crystalline phase).

- Possible method(s): Atomic force microscopy, transmission electron microscopy, scanning electron microscopy, x-ray diffraction.

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18. Lidén, G., "Dustiness Testing of Materials Handled at Workplaces," Annals of Occupational Hygiene, 50, 437–439 (2006).

19 Maynard et al., J. Toxicol Environ. Health, A 67, 87-107(2004)

Representative Electron Microscopy (TEM) picture(s)

This element must be **addressed**.

TEM pictures will provide a qualitative view of the state of a sample of nanomaterials. For example, the perspective will include whether nanomaterials are aggregated and if there is uniformity or dispersity of particle sizes. High resolution scanning electron microscopy (SEM) can provide equivalent information in many cases.

- Possible method: TEM, SEM

Particle size distribution – dry and in relevant media

This element must be **completed**. For information on media considerations, see Annex II.

Definition of particle size: The physical dimensions of the smallest discrete form of a substance under specified measurement conditions. If a group of particles are of differing sizes they may described by a Particle Size Distribution.

Specific surface area

This element must be **completed**. See Annex II for additional information on the term Surface Area.

Specific surface area is highly relevant for a number of parameters for toxicological and ecological risk assessment. It will dictate the surface charge density in cases where nanomaterials are surface functionalised. This in turn has direct consequences on (a) nanomaterial interaction (i.e., agglomeration) with other naturally occurring particulate matter (i.e., contaminant vectors); (b) route of exposure as a function of surface ligand-biological interface (i.e., bioaccumulation pathway, bioavailability); and (c) mechanisms of toxicity (e.g., dose response curves normalised for surface area may indicate different results compared to results presented on a per mass basis). The measured specific surface area is not necessarily biologically available. Porosity data should be considered as well.

- Possible method(s): Brunauer, Emmett, and Teller method[20] ; ISO 9277:1995

Zeta potential (surface charge)

This element must be **completed.** See Annex II for additional information on the term Surface Charge and for information on media considerations.

- Zeta potential plays a key role in determining (1) the degree of colloidal interaction which is itself a function of the pH and ionic strength of the bulk solution; and (2) bioavailability of a compound when considering mass transport through charged membranes as related to exposure.

- Possible method(s): Measure electrophoretic mobility and calculate zeta potential

[20.] http://en.wikipedia.org/wiki/BET_theory

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Surface chemistry, where appropriate

This element must be **completed**. See Annex II for additional information on the term **Surface Chemistry**.

The various modifications of the surfaces of nanomaterials will lead to numerous potential interactions and will play a key role in determining: i) fate in natural aqueous systems; ii) colloidal stability; iii) exposure. A given modification can affect other physical-chemical properties, such as agglomeration, dustiness, zeta potential, surface area, water solubility.

- Possible method(s): Chemical methods that compare the un-functionalised material with the functionalised material. Physical methods such EELS (electron energy loss spectroscopy), and, depending on the dimensions of the particles, possibly XPS (ESCA) or Auger electron spectroscopy**.** Functionalised probe AFM might also be possible and could allow the location of the functionalisations to be determined. More particle methods are ash content, TGA, TG-MS, total carbon content.

Photocatalytic activity

This element must be **addressed**.

Measuring photocatalytic activity will give an indication of the potential for transformations in the environment which in turn represents an important point of concern when evaluating the full life-cycle of the nanomaterial.

Note that UV-B exposure decreases rapidly at increasing depths in the water column. Water and the impurities in it strongly absorb and scatter incoming UV-B radiation. Some substances that are dissolved in water, such as organic carbon from nearby land, will also absorb UV-B radiation and enhance protection of microorganisms, plants, and animals from UV-B. Different masses of water at different locations contain different amounts of such dissolved substances and other particles, making evaluation of UV damage very difficult.[21]

- Possible method(s):
  - Photoreactivity of ZnO has been measured under constant focused photon flux from a 500 W medium pressure mercury arc lamp. The photoactivity index is measured as the zero order rate of the photocatalytic oxidation of liquid propan-2-ol to propanone under oxygenated conditions. Photoreactivities are expressed in terms of moles converted per gram of particulate per hour of irradiation.[22]

Note that ISO TC 206/WG37, Fine ceramics – Test methods for photocatalytic material, has a number of work items on this subject.

Pour density

This element must be **addressed**.

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21. http://earthobservatory.nasa.gov/Features/UVB/uvb_radiation3.php

22. For example, see discussion in SCCNFP/0649/03 *The Scientific Committee On Cosmetic Products And Non-Food Products Intended For Consumers Opinion Concerning Zinc Oxide* http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out222_en.pdf

Pour density is the apparent density of a bed of material formed in a container of standard dimensions when a specified amount of the material is introduced without settling.

- Possible method(s): To be determined (TBD). CEN/TC 184 and ISO TC 206 may publish relevant methods including NP0702: Fine ceramics (advanced ceramics, advanced technical ceramics) - Determination of bulk density of ceramic powders: Part. 2 Untapped density. ASTM D1513-05 to determine the Pour Density of Carbon Black may also be informative..

Porosity

This element must be **addressed**.

Porosity can directly affect the fate of nanomaterials in the environment (i.e., density, colloidal stability) by, for example, modifying the settling velocity in either a bulk solution phase or air phase. An additional consideration while exploring this endpoint is that, in addition to the basic large surface area provided by nanomaterials, a high porosity may permit the nanomaterials to act as vectors for other contaminants, such as heavy metals.

- Possible method(s): ISO 15901 Part 1(mercury porosimetry) and Part 2 (mesopore analysis by gas adsorption), Part 3 (micropore analysis by gas adsorption),, Dye absorption

Octanol-water partition coefficient

This element must be **addressed**.

Octanol-water partition coefficient has found applicability for soluble substances (and indicates the degree of hydrophobicity or hydrophilicity). While not commonly applied to insoluble materials, there has been some utility demonstrated in the case of fullerenes. In the cases where water solubility exists, this is due to colloidal stability, and as such is undefined in non-aqueous phases. Research must address to what degree colloidally suspended particles in the aqueous phase can also be suspended in a non-aqueous phase (such as octanol) and any relevance this may play to partitioning into biologically relevant lipid rich phases, such as cell walls, fat tissue, or other biological systems. See Annex II for additional information on the term **Solubility/Dispersibility**.

- Possible method(s): OECD TG107 *Partition Coefficient (n-octanol/water): Shake Flask Method*, TG123 *Partition Coefficient (1-Octanol / Water): Slow-Stirring Method*

Redox potential

This element must be **addressed**.

- Possible Method(s): Electrochemical experiments with electrode and potentiometer

Radical formation potential

This element must be **addressed**.

Potential to induce free radicals in organisms as has been demonstrated for a number of nanomaterials may have relevance to the toxicity of a MN. This can be measured by various means for different biological systems. Several known pathways have previously been reported in the literature for nanomaterials.

- Possible method(s): **TBD**

Other relevant Physical-Chemical Properties and Material Characterisation information (where available)

This element must be **addressed**. For information on media considerations, see Annex II.

Environmental Fate

Dispersion stability in water

This element must be **addressed**.

The term "dispersion stability" encompasses a number of considerations, with the ultimate goal of understanding which compartment the material will reside in if released into the aquatic environment. The Sponsor should first determine whether the nanomaterial will disperse or partially disperse in water. If the nanomaterial disperses, the Sponsor needs to determine whether the integrity of the particle can be sustained in an aquatic environment (e.g., will it dissolve/degrade). Secondly, for materials that do not readily dissolve/degrade, Sponsors will be asked to determine whether it will likely remain as: i) a uniform dispersion; ii) water suspension; iii) form a film on the surface; or iv) form an aggregate or agglomerate which will settle to sediment. For more information on media considerations, see Annex II.

Degradation, transformation and persistence of MNs in the environment depends on their chemical composition, of both core and surface material. It is likely that many if not most will be relatively persistent in their original particulate form. Although, the organic coatings could be degraded or transformed by environmental factors, there is a lack of data in this area.

- Possible Method(s): **TBD**

Biotic degradability

This element must be **addressed**.

A number of OECD biotic degradation test methods are available for consideration. However these OECD methods have been developed and validated principally for organic compounds. The nanomaterials addressed now under this test programme are principally inorganic; indeed even carbon-based nanomaterials tend to be of an inorganic nature. Hence they will most likely be considered persistent against biodegradation. Consequently a number of considerations exist when employing these studies including: i) biotic degradation studies which employ the test material as an energy source (for example, OECD 301), should be limited to carbon based nanomaterials, and ii) in biotic studies which have a nutrient source, degradation will need to be addressed in terms of directly measuring degradation of the particles and/or appearance of degradation products, rather than assessing production of carbon dioxide or oxygen consumption(mineralisation).

Development of the DDP must include a discussion of which OECD biotic degradation methods are considered appropriate, as well as how best to measure the biotic degradability of particular nanomaterials. Biotic degradation approaches include:

- Ready biodegradability: OECD TG301 Series and OECD TG310 *Ready Biodegradability - CO2 in sealed vessels (Headspace Test)*
- Inherent biodegradability: OECD TG302 Series

In addition, biotic degradation tests simulating a range of environmental compartments are also available including:

- Simulation testing on ultimate degradation in surface water: OECD TG306 *Biodegradability in Seawater*, TG309 *Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test*
- Soil simulation testing: OECD TG304A *Inherent Biodegradability in Soil* and OECD TG 307 *Aerobic and Anaerobic Transformation in Soil*
- Sediment simulation testing: OECD TG308 *Aerobic and Anaerobic Transformation in Aquatic Sediment Systems*
- Sewage treatment simulation testing: OECD TG303A *Simulation Test – Aerobic Sewage Treatment : Activated Sludge Units* and TG303B *Simulation Test – Aerobic Sewage Treatment : Biofilms*

Anaerobic degradation simulating sediments and anaerobic effluent treatment could be tested by:

- OECD TG311 *Anaerobic Biodegradability of Organic Compounds in Digested Sludge: by Measurement of Gas Production*

Identification of degradation product(s)

This element must be **addressed**.

It is important to understand whether an observed effect may be due to a product of degradation and not the test substance itself.

- Possible Method(s): **TBD**

Further testing of degradation product(s) as required

This element must be **addressed**.

- Possible Method(s): **TBD**

Abiotic Degradability and Fate

This element must be **addressed**.

- Possible Method(s):
  - Hydrolysis, for surface modified nanomaterials: OECD TG111(Hydrolysis as a function of pH)
  - Phototransformation: OECD TG316 (Phototransformation of Chemicals in Water - Direct Photolysis)

Adsorption-Desorption

This element must be **addressed**.

- Possible Method(s): OECD TG106 *Adsorption/Desorption Using a Batch Equilibrium Method*

Adsorption to soil or sediment

This element must be **addressed**.

- Possible Method(s): **TBD**

Bioaccumulation potential

This element must be **addressed**.

It is important to develop empirical information on the ability of nanomaterials to concentrate in biological tissue. There is currently no means of predicting bioaccumulation potential; approaches to predicting bioaccumulation potential in the chemical assessment field may not have application to nanomaterials. The challenge to assess bioconcentration or bioaccumulation of MNs is again the detection and characterisation the MNs during the exposure and in the tissues during testing. Possible methods would be.

- OECD TG305 *Bioconcentration: Flow-through Fish Test*, especially including dietary uptake.

Standard BCF testing protocols such as OECD 305 (OECD 1996) may have critical limitations in sole testing of bioaccumulation of nanoparticles. It is likely that in most cases the relatively large size of nanoparticles compared to macromolecules limits their uptake by fish compared to standard molecular chemical substances. It has been observed that a large molecular size effectively limits direct uptake of large molecules (> 0.5 nm cross diameter). It is therefore unlikely that the existing standard protocol OECD 305 is suitable for these particles.

- OECD TG 315 *Bioaccumulation in Sediment-dwelling Benthic Oligochaetes Bioaccumulation in sediment worms*

The sediment method OECD TG315 could be especially relevant as sediment has been debated to act as a sink for NMs in the aquatic environment.

Fish dietary BAF testing is not a standard OECD testing protocol yet. This spiked food method is suitable for testing of poorly soluble large molecules and might be suitable for testing several classes of nanoparticles, either entirely or in combination with the OECD 305. However, more data using a harmonised OECD dietary protocol, especially for testing nanomaterials, are needed.

Other relevant environmental fate information (when available)

This element must be **addressed**.

Other information relevant to understanding potential environmental fate of the sponsored MN should be provided, if available.

Toxicological and ecotoxicological effects

While in most cases the determination of an adverse outcome is sought through dose selection for many of the tests described for environmental and mammalian toxicity described in this guidance, Sponsors should note that non-adverse results are also informative for exploratory testing of Phase 1, and these should be reported.

Additional Material Characterisation Considerations

Sponsors conducting toxicity tests should use test material that has been characterised in accordance with the elements under "Nanomaterial Information/Identification" and "Physical-Chemical Properties and Material Characterisation" of Annex I and in the reporting template (see Page 47). Transportation, handling, and storage conditions may affect the characteristics of some MNs. Therefore, sponsors should describe plans to evaluate the effects of such conditions in the DDP. Best handling conditions should be described and DDPs should set procedures to determine whether and when the test material will need to be re-characterised prior to initiating toxicity tests.[23] Such re-characterisation may be required either partially or fully with respect to the elements in Annex I. Note that in some cases it may be determined through such evaluations that re-characterisation will need to be done even in batches or aliquots used at different dosing points in the same test procedure. Such decisions will likely depend on the nature of the material and the conditions surrounding its transport, handling, and storage. Note, however, that it is not expected that known catalytic activity, photocatalytic activity, pour density, porosity, K_{ow}, redox potential, and radical formation potential will need to be re-characterised. Sponsors may choose to use adequately justified surrogate measures to determine whether or not and at what stage re-characterisation is needed. Surrogate measures could include, for example, "Loss on Incineration" could be proposed for measurement of purity with where organic matter contamination may be an issue for a particular MN.

Environmental Toxicology

In developing an environmental toxicity testing strategy, Sponsors should first undertake an examination of the sponsored MN's physical-chemical properties, as well as results from environmental fate testing. This will assist in determining into which environmental compartment or compartments the nanomaterial will partition, and consequently which series of ecological tests ought to be considered for priority testing.

For example, if it is determined that the nanomaterial is expected to partition solely into the water compartment, then pelagic tests would need to be conducted. However, if it is expected to strongly adsorb, or has a very low water availability, then it may be expected to partition into soil or sediment compartments and therefore ecotoxicity testing would be needed for those compartments. However, regardless of the compartment the nanomaterial is expected to partition, both acute and chronic ecotoxicity testing should be undertaken in order to build mathematical relationships between acute and chronic toxicity for categories of nanomaterials. Surface coating, either manufactured or natural (e.g. humic acids), and aggregation/disaggregation will largely define the fate, behaviour and bioavailability of nanoparticles. Stabilisation by surface coating may maintain nanoparticles within the water column and hence increase bioavailability to aquatic organisms. Aggregation will likely lead to nanoparticles settling and joining the sediments. This could potentially make benthic organisms key receptors and targets of nanoparticles.

A similar strategy should be applied when examining toxicity to microorganisms. If the properties indicate that the substance would be expected to partition into the soil, then microbial toxicity testing relating to nitrogen fixation or carbon transformation may be necessary. However, if it is expected to partition readily to the sediment, then the impact to anaerobic microorganisms should be considered.

[23] Other means of assuring no changes may be developed as well. For example, a Sponsor may establish procedures that have been demonstrated to not affect particular properties, and refer to the use of those procedures in lieu of re-characterisation.

Effects on pelagic species (short term/long term)

- Fish: OECD TG203 *Fish, Acute Toxicity Test*, TG204 *Fish, Prolonged Toxicity Test: 14-Day Study*, TG210 *Fish, Early-Life Stage Toxicity Test*, TG212 *Fish, Short- term Toxicity Test on Embryo and Sac-fry Stages*, TG215 *Fish, Juvenile Growth Test*

- Crustaceans: OECD TG 202 *Daphnia sp. Acute Immobilisation Test*, OECD TG 211*(Daphnia magna Reproduction Test*

- Algae: OECD TG201 *Freshwater alga and cyanobacteria growth inhibition test*

Effects on sediment species (short term/long term)

The assessment of effects on the sediment organisms might be especially relevant when evaluating MNs as benthic organisms are considered to relevant target organisms if the MNs are concentrating in sediment after aggregation.

- Possible Method(s):

  - OECD TG218 Sediment-Water Chironomid Tox Using Spiked Sediment, OECD TG219 Sediment-Water Chironomid Tox Using Spiked Water

  - OECD TG 225 *Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment*. This method could be especially interesting as the same organism could be used for bioaccumulation studies (OECD TG 315)

Effects on soil species (short term/long term)

This element must be **addressed**.

- Possible Method(s): OECD TG207 *Earthworm, Acute Toxicity Tests*, TG222 *Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)*, and OECD TG220 *Enchytraed reproduction test*

Effects on terrestrial species

This element must be **addressed**.

- Possible Method(s):

  - Terrestrial/soil. The ISO springtail reproduction test with *Folsomia candida*. ISO guideline No. 11267 measures the effects of toxicant exposure on the survival, growth and reproduction of the soil-dwelling springtail *Folsomia candida*. The test has been standardised by the International Standards Organisation and has been extensively ring-tested prior to approval for regulatory testing. To date, the standardised springtail test has been used for toxicity assessment of many metals, as well as a range of organic chemicals[24] during which responsiveness and robustness of the test for assessment of chronic toxic effects on ecologically relevant traits have been satisfactorily established. The corresponding OECD test guideline is currently under finalisation.

[24] see Hopkin, 1997; Fountain and Hopkin, 2005

- Terrestrial/soil. OECD TG207 *Earthworm, Acute Toxicity Tests,* TG222 *Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)* and Enchytraeid Reproduction Test, OECD TG220. These three tests measure the toxicity of chemicals on survival for the acute test and weight change, feeding activity and reproduction for the chronic test both using the earthworms *Eisenia fetida* and *Eisenia andrei and* Enchyttraed worm *Enchytraeus albidus*. The procedures were both developed by the ISO and OECD and have each been ring tested and approved for regulatory testing. To date, the two standardised earthworm toxicity tests have been widely used for regulatory assessment of the non-target effects of pesticides, as well as in studies of the toxic effects of metals and organic chemicals.[25] This work has established these tests as sensitive, reliable and robust.
- Predatory mite test OECD TG 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil. This method assesses the effects of test material on terrestrial predatory animals.

Effects on microorganisms

This element must be **addressed**.

- Possible Method(s): OECD TG216 *Soil Microorganisms: Nitrogen Transformation Test*, OECD TG217 *Soil Microorganisms: Carbon Transformation Test*

Effects on activated sludge at WWTP

- OECD TG209 *Activated sludge respiration inhibition test*

Other relevant information (when available)

Other information that should be reported if available includes data obtained through alternative methods using *in vitro* models or assays using a reduced number of animals, structure activity relationships; and mechanistic information.

Mammalian Toxicology

Sponsors should consider likely exposure process to prioritise testing approaches and test method choices.

Pharmacokinetics/Toxicokinetics (ADME)

This element must be **addressed**.

Adsorption, Distribution, Metabolism and Excretion (ADME) assessments have found great value in understanding material distribution throughout the body of surrogate species for humans, and the data can indicate potential target organs for systemic toxicity experiments. ADME information will be of great value for nanomaterials, but may be very challenging to obtain. It is expected that the Sponsor will not only characterise the nanomaterial in the dosing solution, but that the nanomaterial will be characterised in the biological matrix (e.g., protein binding). Methods that have been used to assist in tracking materials in ADME experiments include radiolabeling or chemical tags. The Sponsor must include an analysis of any modifications and will be expected to present data to document any differences

[25] Spurgeon et al., 2003

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these tagging methods may have on ADME parameters. For example, would using a radiolabel itself cause a toxicological impact?

- Possible Method(s): **TBD**

Acute toxicity

This element must be **addressed**.

Acute toxicity tests should explore the types of toxicity that may occur for brief exposures to the sponsored MN. The routes of exposure should include oral and dermal administration, but may include inhalation for some nanomaterials. The dossier should consider the utility of *in vitro* test methods where appropriate in addition to *in vivo* toxicity tests. Acute toxicity tests typically use both sexes of at least two species to monitor for enhanced responses in a particular sex/species.

- Possible Method(s): OECD TG420 *Acute Oral toxicity – Acute Toxic Class Method*, TG423 *Acute Oral Toxicity – Acute Toxic Class Method*, TG425 *Acute Oral Toxicity: Up-and-Down Procedure*, TG402 *Acute Dermal Toxicity,* TG403 *Acute Inhalation Toxicity*, additional short-term inhalation test. 5-day Inhalation study with 28/90 day post-exposure monitoring and periodic BAL.

Possible methods and approaches to consider in developing plans to address acute toxicity include:

a) Skin Corrosion

- Possible Method(s): An OECD method has been established for acute skin corrosion test using in vitro methods (OECD TG430 In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test (TER), TG431 In Vitro Skin Corrosion: Human Skin Model Test, TG435 In Vitro Membrane Barrier Test Method for Skin Corrosion. These tests are meant to replace in vivo testing of skin corrosivity, and the dossier should explain where the Sponsor believes these tests will or will not allow accurate determination of the corrosive nature of the sponsored MN.

b) Skin Irritation

- Possible Method(s): (OECD TG404 Acute Dermal Irritation/Corrosion).

c) Skin sensitisation

- Possible Method(s): Methods have been established and validated for a number of chemicals using the guinea pig skin sensitisation model (OECD TG406 *Skin Sensitisation*) and the murine local lymph node assay (OECD TG429 *Skin Sensitisation: Local Lymph Node Assay*). The latter (OECD TG429) is a more recently evaluated assay, and is considered to be more accurate and utilises fewer animals than previous assays; however, penetration by insoluble materials is problematic and could generate false negatives in this assay. The dossier should explain the test to use, and describe any modifications to the OECD assays that are deemed necessary regarding nanomaterial testing (e.g., subcutaneous injections as modification to OECD TG429 to introduce insoluble materials should be considered).

d) Acute Eye Irritation

- Possible Method(s): OECD 405 (Acute Eye Irritation/Corrosion) should be used only if the nanomaterial has not shown evidence of skin corrosivity. There are currently no *in vitro* eye irritation studies. The dossier should contain a description of the sponsored MN characterisation before and after dosing with this method.

e) Phototoxicity

- Possible Method(s): This toxicity occurs when a material interacts with light and generates a new product or catalyzes the formation of other products that are toxic. An OECD method exists for acute phototoxicity using cultured murine cells (OECD TG432 *In Vitro 3T3 NRU Phototoxicity Test*).

Repeated dose toxicity

This element must be **addressed**.

Repeated dose toxicity studies allow an exploration of cumulative effects of materials on biological systems. These studies should be informed by acute toxicity studies as well as by likely exposure scenarios for the sponsored MN. Many of the repeated dose toxicity studies are described in OECD test guidelines. The dossier should incorporate description of expected nanomaterial distribution and fate (disposition in the body) during a repeated dose study and the rationale for and approach to target specific organ system evaluations (for example, sponsored MN characterisation in and histopathology of selected organs).

Possible routes and approaches to consider in developing plans to address repeated dose toxicity include:

a) Oral

- Possible Method(s): OECD TG407 *Repeated Dose 28-Day Oral Toxicity Study in Rodents* and OECD TG409 *Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents* are available for repeated oral dosing studies in rodents and in non-rodents. Sponsors should include repeated dose toxicity studies via the oral route for both sexes of mice and rats for the 28-day studies, and a description of expected 90-day repeated dose studies in rodents. Any reduction of species or sexes in the 90-day studies should be described in the dossier. Typically, these studies include complete histopathology of tissues to document organ response (or lack thereof) to the test articles. There are currently no validated *in vitro* methods to address repeated dose toxicity studies; however, the Sponsor should consider companion *in vitro* methods to explain any toxicity detected in the *in vivo* studies (e.g., membrane transport in specific cells).

b) Dermal

- Possible Method(s): OECD TG410 *Repeated Dose Dermal Toxicity: 90-Day* is available for repeated dermal dosing studies in rodents and in non-rodents. Toxicokinetic and repeated dose oral studies can provide rationale for not conducting dermal toxicity studies; however, the Sponsors should provide an explanation why repeated dose dermal toxicity studies should not be used.

c) Inhalation

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This area of toxicity has received the most attention due to the potential airborne transport of nanomaterials and likelihood of inhalation during manufacture or use. For example, toxicity test data obtained by inhalation exposure experiments are used for the hazard characterisation and for setting exposure limit values in the workplace.

- Possible methods: OECD TG411 *Subchronic Inhalation Toxicity: 90-Day,* OECD TG412 *Repeated Dose Inhalation Toxicity: 28/14-Day*, OECD TG413 *Subchronic Inhalation Toxicity: 90-Day* are available for repeated dose inhalation studies.

If available, the results following tests must be included in the dossier for Phase 1[26]:

Chronic toxicity

The dossier must describe any relevant existing chronic study results of the sponsored MN (e.g., results from toxicokinetics and repeated-dose toxicity studies) including carcinogenicity studies. The dossier should report the routes of administration, frequency of dose, certification and characterisation of nanomaterial, any early endpoints to accompany the chronic toxicity test (e.g., micronucleus test at 1 year), and a list of all tissues histopathologically examined at the end of the study. Consistency of the reported results to OECD TGs 451, 452, 422, and 453 should be considered, and the dossier should contain any descriptions of the study that are different from these guidelines.

Reproductive toxicity

The dossier must describe relevant reproductive toxicity test results for the sponsored MN. In particular, test results should be considered in context of toxicokinetics data and understanding for the nanomaterial with respect to distribution of the sponsored MN to reproductive organs or the developing fetus (i.e., transplacental transfer) or neonate (i.e. mammary transfer) occurs.

Examples of studies that would be relevant if available for the sponsored MN would include:

a) One-generation reproductive toxicity study which involves exposure of the male and female rodents for a specified length of time prior to mating (OECD TG415 *One-Generation Reproduction Toxicity*). Dosing of the pregnant females is continued through parturition and until weaning of the offspring. These studies are typically conducted with oral administration of test article; however, other routes of exposure could be used. The dossier should contain a description of the route of exposure, nanomaterial characterisation in the dose solution, on-going test parameters (e.g., fertility rate, successful offspring, body weights), post-mortem examination (i.e. tissues to be examined histopathologically), any dose metrics (i.e., detection of nanomaterials in tissues), and any accompanying tests (e.g., micronuclei formation in dams).

b) A modification of the one-generation reproductive toxicity test is exposure of the dam only to the test article from conception to birth (OECD TG414 *Prenatal Developmental Toxicity Study*).

c) A two-year (or additional generations) reproductive toxicity test (OECD TG416 *Two-generation Reproduction Toxicity Study*) extends the exposure of the offspring from the one-generation test through maturation, mating and production of a second generation of offspring (F2). The same considerations as mentioned above should be included in the dossier regarding a two- (or multiple) generation reproductive toxicity test.

[26] Note that plans to test for these endpoints would generally be covered in DDPs and guidance for Phase 2 testing. It is not the intention of WPMN that the following endpoints must be addressed through testing undertaken in Phase 1 testing.

Developmental toxicity

- Possible Method(s): OECD TG414 *Prenatal Developmental Toxicity Study*, TG421 *Reproduction/Developmental Toxicity Screening Test*

Genetic toxicity

The dossier must describe any relevant existing genetic toxicity test results for the sponsored MN. Examples of studies that would be relevant if available for the sponsored MN would include:

a) *In vitro* Genotoxicity

The dossier should explain which tests have been used, the expected utility of the test, and characterisation of the sponsored MN during the test (e.g., did particle size change when added to culture).

- Possible Method(s): *In vitro* methods are available for bacterial genotoxicity (*S. typhimurium* and *E. coli*; OECD TG471 *Bacterial Reverse Mutation Test)*, mammalian cell chromosome aberrations (OECD TG473 *In vitro Mammalian Chromosomal Aberration Test*), and mammalian cell gene mutations (mouse lymphoma cells; OECD TG476 *In vitro Mammalian Cell Gene Mutation Test*).

b) *In vivo* Somatic Cell Genotoxicity

Typically, *in vivo* tests are conducted when a material has been shown to be genotoxic in *in vitro* tests; however, the dossier should explain why *in vivo* somatic cell genotoxicity tests should not be conducted in rodents being tested for repeated dose toxicity tests. The dossier should describe the test results available or to be conducted, whether these will be on animals as part of another test (e.g., 90-day oral repeated dose), and describe any *in vivo* additional somatic tests that are available or will be conducted.

- Possible Method(s): OECD guidelines exist for induction of chromosomal aberrations in rodent bone marrow cells (OECD TG475 *Mammalian Bone Marrow Chromosomal Aberration Test*), the induction of micronuclei formation in peripheral blood and bone marrow erythrocytes (OECD TG474 *Mammalian Erythrocyte Micronucleus Test*), and the induction of unscheduled DNA synthesis (i.e. DNA repair) in the liver of treated animals (OECD *TG486 Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo*).

c) *In vivo* Germ Cell Mutagenicity

The OECD has approved several methods for evaluating germ cell mutagenicity; however, the WPMN has recommended that germ cell mutagenicity assays not be conducted if somatic cell genotoxicity tests are conducted.

Experience with human exposure

The Dossier must describe relevant experience with human exposure to the sponsored MN. Sponsors are encouraged to coordinate the selection of sponsored MNs and subsequent evaluation of relevant experience with human exposure with the WPMN project *Co-operation on Exposure Measurement and Exposure Mitigation* (SG8).

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Other relevant test data

Other toxicity tests or endpoints exist that may improve understanding of the properties of the sponsored MN. Sponsors are encouraged to consider these studies which improve interpretation of standard toxicity tests. Some of these to consider are photocarcinogenicity, start-stop studies, and biomarkers of exposure or toxicity (e.g., genomics, proteomics, metabonomics), data obtained through alternative methods using *in vitro* models or assays using a reduced number of animals, structure activity relationships; and studies to investigate potential mechanisms of toxicity.

Material Safety

If available, the results following tests must be included in the dossier:

a) Flammability
   - Possible Method(s): TBD

b) Explosivity
   - Possible Method(s): TBD

c) Incompatibility
   - Possible Method(s): TBD

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SECTION 3: OUTPUTS

The Working Party has identified seven principal outputs from the WPMN testing programme. Each of these outputs is discussed below.

1. Phase 1 Data

The test programme, particularly Phase 1, seeks to develop a better understanding of the properties of representative MNs through study of sponsored MNs. As a general consideration, both positive and negative results (for example, with regard to hazard) are useful in developing this understanding. Therefore, all study results should be reported.

Phase 1 testing results should be reported using the reporting template approved by the WPMN. Such template is being developed by the WPMN to be made available for sponsors in a later stage. The template will be organised around agreed-upon reporting elements. However, Sponsors are not limited to these reporting elements—they also may report other information that they believe is important to describing the findings of their testing programme. The reporting template is distinguished from Dossiers. A Dossier includes an interpretive compilation of data[27] that is derived from the reported templates. A Dossier may include descriptions of different materials including MNs and comparison materials and may includes different tests in one endpoint, whereas the reporting template would tend to report one test and one material for each entry.

2. Recommendations for Phase 2

Each Sponsor should communicate to the Working Party its recommendations for any Phase 2 testing that it believes should be conducted. Such recommendations can be made at any point in the testing programme, but in all cases should be described at the conclusion of Phase 1 testing. In its recommendation, the Sponsor should indicate whether it will conduct some or all of the Phase 2 testing, or whether it recommends that some or all of the testing be done by another entity.

3. Phase 2 Data (if developed)

See guidance for reporting Phase 1 data.

[27] Data includes both generated data and existing data.

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4. Characterisation of Findings

In the DDP submitted to the 8th WPMN and in the Final Phase 1 WPMN Testing Programme Report, as well as after completing Phase 2 testing if it is conducted, the Sponsor should characterise the findings of the testing programme. While they will differ depending on the materials and endpoints testing, such characterisations of findings should address the following considerations, as well as any other considerations the Sponsor wishes to include:

- The approaches used for testing, including what guidelines, methods, and protocols were followed, as well as what other plausible approaches, if any, were considered but not used. For examples, if an OECD test guideline was not used, what considerations were given in the selection of another approach?
- The assumptions made in pursuing testing approaches. For example, were certain solvents or other materials used (or not used) in testing based on previous experience with those materials?
- Conclusions or inferences that can be made from endpoint-specific test results and how those conclusions or inferences relate to findings or inferences from findings of other tests within the testing programme as well as other tests conducted outside the testing programme. For example, did what was learned in an aggregation/accumulation study in water affect the approach taken to aquatic species toxicity testing done within the testing programme, and how does this relate to similar aggregation/accumulation and/or aquatic species toxicity testing done outside the testing program?
- Data gaps that remain for a given material and/or endpoint, and whether the testing group has recommendations for priorities in filling those data gaps. For example, if upon conclusion of testing the Sponsors believe that gaps remain in understanding what specific material properties impact toxicity, do the Sponsors have recommendations as to which particular properties should be the focus of future testing?
- Description of uncertainties in the findings. For example, if a mammalian toxicity test yielded an adverse effect that is common in the mouse but not the rat, what are the uncertainties of extrapolating those results to humans?

It is also important that the Working Party write a report that characterises the overall findings from the WPMN testing programme. Such characterisation should be done at specific points in the testing programme, as part of the Working Party's communication strategy, as well as in the Final Phase 1 WPMN Testing Programme Report.

5. Test Guidelines and Methods Report

Since an important Working Party objective is to identify issues related to the applicability of OECD harmonised test guidelines to MNs, each Sponsor should develop a summary of which OECD Test Guidelines were used in the testing programme, any problems that were encountered in using those test guidelines, what other methods were used and why (as described in 4 above), and any other test guidelines considerations that the Sponsors believe are important to communicate.

Furthermore, at the earliest practical point in development of modifications to OECD Test Guidelines, Sponsors should communicate with OECD through the clearinghouse function set up for this testing programme, and with the WNT as necessary, so that coordination of the development of modifications of Test Guidelines can be assured. This communication will tend to draw upon similar development efforts undertaken by Sponsors of other sponsored MNs and more directly move methods development toward standardised approaches for MNs.

6. Communication and Interim Progress Report

Each Sponsor should consider when, over the course of its testing programme, it should communicate with other Sponsors as well as other interested parties and stakeholders in order to share information and take advantage of developing a common understanding from consideration of other testing programmes. Sponsors should explore various possible communication means, including:

- Entry of published research or other publicly released information into the WPMN database.
- Use of wiki technology to post DDPs and associated changes on the WPMN password-protected website: with revision rights limited to sponsors
- Communication at WPMN meetings as well as through the Secretariat between meetings.
- Making interim results available according to the WPMN's communication strategy.
- By holding state-of-the-science workshops or conferences, or by leveraging venues outside the WPMN to present and discuss interim or final test findings.

7. Final Phase 1 WPMN Testing Programme Report

Phase 1 final products will include:

- **Individual Dossiers.** Final dossiers will be published on the OECD public web site. Data will be reported in robust summaries.

- **Final Report.** It is important that the Working Party issue a report that aggregates and summarises approaches, findings, and recommendations from the various Sponsors. Discussions on the format and content of such a report should occur over the course of the testing programme.

8. Outputs Related to the Scientific Findings of a Project (Publications)

Knowledge resulting from the safety testing (Phase 1 or 2) of a sponsored MN can be published in the scientific literature if deemed necessary by the Sponsors of the MN.

Lead Sponsors, Co-Sponsors and Contributors of a sponsored MN are responsible for co-ordinating with each other when producing publications. For example, Sponsors need to agree on the way they publish and on a fair redistribution of the impact of those publications among themselves.

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ANNEX I.
LIST OF MANUFACTURED NANOMATERIALS AND LIST OF ENDPOINTS FOR PHASE ONE OF THE OECD TESTING PROGRAMME

This annex has been extracted from the OECD Publication, *List of Manufactured Nanomaterials and List of Endpoints for Phase One of the OECD Testing Programme* [ENV/JM/MONO(2008)13/REV], which is available for free download at: www.oecd.org/env/nanosafety .

LIST OF REPRESENTATIVE MANUFACTURED NANOMATERIALS FOR TESTING

The list of representative manufactured nanomaterial has been selected by the WPMN for use in its work. The word "representative" refers to those manufactured nanomaterials now, or soon to enter into commerce, for inclusion in a set of reference materials to support measurement, toxicology and risk assessment of nanomaterials. Therefore, the list was mainly selected taking into account those materials which are in commerce (or close to commercial use), but other criteria were also considered: for example, production volume, the likely availability of such materials for testing and the existing information that is likely to be available on such materials.

It was also emphasised that certain nanomaterials not included in the list may become important in the future and certain nanomaterials currently on the list may have (over time) reduced production and/ or use. Accordingly, the list should be considered as a "snapshot in time", of those nanomaterials in commerce or likely to enter into commerce in the near term. At the same time, some nanomaterials on the list may have variants[1] that the WPMN may wish to consider in detail in the future.

Nanomaterials

- Fullerenes (C60)
- Single-walled carbon nanotubes (SWCNTs)
- Multi-walled carbon nanotubes (MWCNTs)
- Silver nanoparticles
- Iron nanoparticles
- Carbon black
- Titanium dioxide
- Aluminium oxide
- Cerium oxide
- Zinc oxide
- Silicon dioxide
- Polystyrene
- Dendrimers
- Nanoclays

The order in which the nanomaterials are listed above does not indicate a priority.

[1] For example, C60 could be broadened to other fullerenes as well as chemically modified varieties of C60; it may also be important to analyse chemically modified single- and multi- walled CNTs; and the influence of surface coatings of elemental and metal oxide nanomaterials, and/ or their different shapes – e.g., rods.

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LIST OF ENDPOINTS FOR PHASE ONE TESTING

The list of endpoints is a set to take into account, when testing specific sponsored MNs for human health and environmental safety within phase one of the Testing Programme. Addressing this set should ensure consistency between the various tests to be carried out on specific nanomaterials. It should also lead to the development of dossiers for each nanomaterial describing basic characterisation, fate, ecotoxicity and mammalian toxicity information.

It is also expected that the list of endpoints be refined based on the practical results obtained through the testing programme. As such, phase one testing is expected to be of an exploratory nature, science-based and without any consequences for existing regulatory datasets.

Endpoints

Nanomaterial Information/Identification

- Nanomaterial name (from list)
- CAS Number
- Structural formula/molecular structure
- Composition of nanomaterial being tested (including degree of purity, known impurities or additives)
- Basic morphology
- Description of surface chemistry (e.g., coating or modification)
- Major commercial uses
- Known catalytic activity
- Method of production (e.g., precipitation, gas phase)

Physical-Chemical Properties and Material Characterisation

- Agglomeration/aggregation
- Water solubility
- Crystalline phase
- Dustiness
- Crystallite size
- Representative TEM picture(s)
- Particle size distribution
- Specific surface area
- Zeta potential (surface charge)
- Surface chemistry (where appropriate)
- Photocatalytic activity
- Pour density
- Porosity
- Octanol-water partition coefficient, where relevant
- Redox potential
- Radical formation potential
- Other relevant information (where available)

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Environmental Fate

- Dispersion stability in water
- Biotic degradability
  - Ready biodegradability
  - Simulation testing on ultimate degradation in surface water
  - Soil simulation testing
  - Sediment simulation testing
  - Sewage treatment simulation testing
- Identification of degradation product(s)
- Further testing of degradation product(s) as required
- Abiotic degradability and fate
  - Hydrolysis, for surface modified nanomaterials
- Adsorption- desorption
- Adsorption to soil or sediment
- Bioaccumulation potential
- Other relevant information (when available)

Environmental Toxicology

- Effects on pelagic species (short term/long term)
- Effects on sediment species (short term/long term)
- Effects on soil species (short term/long term)
- Effects on terrestrial species
- Effects on microorganisms
- Other relevant information (when available)

Mammalian Toxicology

- Pharmacokinetics (ADME)
- Acute toxicity
- Repeated dose toxicity

If available:

- Chronic toxicity
- Reproductive toxicity
- Developmental toxicity
- Genetic toxicity
- Experience with human exposure
- Other relevant test data

Material Safety

Where available:

- Flammability
- Explosivity
- Incompatibility

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ANNEX II.
DEFINITIONS AND ADDITIONAL GUIDANCE FOR PHYSICAL-CHEMICAL CHARACTERISATION

This annex provides a glossary and guidance of physical chemical characterisations of manufactured nanomaterials. The page numbers associated with the headings indicate the pages in the main text of this Guidance Manual.

The information in this annex builds on a Working Draft "Compilation of Definitions for Selected Physico-chemical Characterisation of Engineered Nanoscale Materials for Toxicologic Assessment" (Version 11-13-2008) of International Organisation for Standardisation, Technical Committee-Nanotechnologies - Working Group 3: Health, Safety and Environmental Aspects of Nanotechnologies - Project Group 5: Technical Report - Guidance on physico-chemical characterisation of engineered nanoscale materials for toxicologic assessment (ISO/TC229 /WG3/PG5). The Technical Report is at preparatory stage at the time of writing. The latest status of the Technical Report is available at the ISO website http://www.iso.org.

Term: Aggregation/Agglomeration State (Pages 30-31)

Definition: 1– A'/A", where A' is the effective cross-section of a primary particle and A" is the effective cross-section of an agglomerated/aggregated particle[29].

Clarification:

Aggregate

Particle comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components. The forces holding an aggregate together are strong forces, for example covalent bonds, or those resulting from sintering or complex physical entanglement. Aggregates are also termed secondary particles and the original source particles are termed primary particles[30].

Agglomerate

Collection of loosely bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components[31].

For this to be the case all primary particles would need to be at the surface of the agglomerate, which is impossible. I think a more accurate statement would be "where the surface area measured by gas absorption – BET – is similar to the sum of the surface areas of the individual components"

Relevance to toxicology/ecotoxicology: Current understanding is that as particles agglomerate or aggregate, the result is secondary particles that are larger, which might affect exposure. For example, if primary particles aggregate or agglomerate, the material might not enter a cell.

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29. Bernhardt, C., Particle Size Analysis: Classification and Sedimentation Methods, Kluwer, 1994.

30 ISO/TC229, Nanotechnologies – Terminology and Definitions for Nano-objects – nanoparticle, nanofibre and nanoplate, ISO/TS 27687 ISO Copyright Office, Geneva, 2007, p. 8.

31 ISO/TC229, Nanotechnologies – Terminology and Definitions for Nano-objects – nanoparticle, nanofibre and nanoplate, ISO/TS 27687 ISO Copyright Office, Geneva, 2007, p. 7.

Measurand: In this method the effective cross-section is the measurand. The effective cross-section can be determined by measuring aerodynamic/light scattering properties or by electron microscopy. For nanomaterials with a non-zero width of the distribution of agglomerated/aggregated particle sizes, the distribution of the degree of agglomeration should be characterised.

See Media Considerations below for additional information.

Term: Composition (Pages 29 & 32)

Definition: Chemical identity and molecular structure of the nanomaterial

Clarification: Composition characterisation must include those substances expected as well as those undesired such as impurities.

Furthermore, the molecules attached to the surface could be considered under composition characterisation. However, we presently list surface chemistry separately.

This point is particularly challenging as the surface molecules, often called capping molecules, are dynamic. They may exchange dynamically with molecules in the solution. Their arrangement, such as lying on the surface versus upright would likely impact toxicity.

From a manufacturing perspective, a product formed from two or more discrete chemical materials (e.g., compounds or elements), which materials are not chemically bonded to each other. An alloy is usually a composition (?), but may in some instances (e.g., intermetallics, etc.) be a compound[32].

Relevance to toxicology/ecotoxicology: For the nanomaterial, composition would include the type of elements involved in the core as well as the form or valance of those elements. Furthermore it would be of value to have the crystalline state of the core such as fcc, hcp, epsilon, etc. (crystal structure would be better dealt with under a separate entry)

Measurand:

- Atomic abundance
- Elemental analysis
- Data may be presented perhaps in ratio or percentage of whole.
- Crystalline state
- Metal valance states (e.g. Iron (Fe) as in Fe_2O_3 vs. Fe_3O_4)

For carbon-containing materials, it may be difficult to determine which allotrope of carbon is present, for instance, nanotube, fullerene, multi-walled CNT or single-walled CNT, etc. If we include surface chemistry, other measurands would need to be added.

This measurand should also include phase composition, such as 10% of amorphous carbon, 10% of graphite, 80% of such and such SWCNT. Please note that this example applies not just to carbon-containing nanomaterials, but for many chemical elements and compounds.

[32] listbox.wipo.int/wilma/ipcreform/200304/msg00001/Glossary_e_Paris_approved.doc

Term: Particle Size/ Size Distribution (Page 33)

Definition: The physical dimensions of particle determined by specified measurement conditions and with a specified method. If a group of particles are of differing sizes, they may be described by a Particle Size Distribution.

Clarification: The conditions to which a substance is subjected may affect the size of the discrete form of a substance. The conditions may affect aggregation/disaggregation; agglomeration/deagglomeration, particle growth, particle dissolution and the like.

The measured size of a particle is always dependent on the particular method that is being used to examine, measure or visualise it. Particle size is measured by assessing effects on physical phenomena where the magnitude of the effect depends on the size of the particle being examined. Examples of different measurement methods are diffusion velocity in liquids, electrophoretic mobility in gases and dynamic light scattering of particles or the integral surface measurement of a particle system (BET method). Any given particle will interact with its environment according to its own specific physical and chemical make-up. This means that the size of a particle reported by one technique might not be the same as the size when measured with another technique. In many fields it has been the custom to define particle size ranges with common behaviour, sources or composition using the method of measurement embedded in the definition. An example is the term ultrafine particles defined as particles with equivalent diameters smaller than 100 nm. Equivalent diameter refers to the practice of reporting the size of a particle of unknown composition or shape as if the particle had known composition and spherical shape. For example, when the particles are measured using an inertial based instrument, aerodynamic diameter is an equivalent diameter, computed as if the particle has unity specific gravity and a spherical shape corresponding to the measured particle settling velocity. Unfortunately the term ultrafine particle is sometimes used interchangeably with the term nanoparticle. Initially, the term nanoparticle was used to describe man-made particles smaller than 100 nm with unique properties[33].

Relevance to toxicology/ecotoxicology: Particle size is a critical parameter in the assessment of environmental, health and safety aspects of nanoscale materials and questions regarding the EHS impacts from nanoscale materials often relate to size. Indeed, relating change in size to changes in other properties might lead to predictable mathematical relationships. The evaluations of EHS impacts necessarily involve biological systems which are themselves complex and may also introduce complications into the evaluation of material properties. Biological systems generally include water, which might encourage an increase in particle size, but might also include biosurfactants that can encourage collections of particle to disperse. Dissolved materials in biosystems may adsorb on or be absorbed by substances potentially affecting particle size and corresponding biological responses. For example, a solid particle that in its dry form is 50 nm may be 1000 nm in biological media.

Addressing this endpoint should include discussion of the effect of particle size on point of deposition in inhalation testing, its potential impact on translocation and penetration of biological barriers, e.g. the blood-brain barrier, its impact on penetration into cells and subcellular structures, dispersion in environmental media, etc., as appropriate.

Measurand: For a representative sample of nanoparticles, determine both average size of individual particles and size distribution of the sample of particles.

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33. ISO/TC229, Nanotechnologies – Terminology and Definitions for Nano-objects – nanoparticle, nanofibre and nanoplate, ISO/TS 27687 ISO Copyright Office, Geneva, 2007, p. 10

After dispersing any loosely bound agglomerates using slow agitation, measure the average size and size distribution of particles using: i) microscopy (particle counting) or ii) dynamic light scattering with/without prior size exclusion chromatography treatment.

Particle size can be also determined through measurements of aerodynamic properties.

See Media Considerations below for additional information.

Box 2: Guidance Information for Particle Size/ Size Distribution

The text below is intended to present an example of the detailed guidance with regard to physical chemical properties. A similar level of guidance may be provided for the other physical-chemical properties and be included in subsequent versions of the Guidance Manual.

Clarification

The conditions to which a substance is subjected may affect the size of the discrete or free form of a substance and include environmental and test organism impacts. The conditions may affect aggregation/disaggregation, agglomeration/deagglomeration, particle growth (e.g. crystal growth, absorption), particle dissolution and the like. In addition, particle size and its distribution will be directly dependant on the physical form of the material under study (i.e., dry, deposited, wet dispersed, aerosol, coated, etc). For the purposes of this guidance, particle size and distribution in all physical forms will need to be considered in water quality and pH ranges that are representative of a variety of environmental conditions and those relevant to human health. Terminology as defined in ISO Technical Specification 27687 09-2008 is included in this definition by reference.

Considerations

The size of a substance that is the subject of an evaluation is a fundamental issue with respect to assessing environmental, health and safety aspects of nanoscale materials. Indeed, relating variation in size to variations in other properties may lead to predictable mathematical relationships. The evaluations of EHS impacts necessarily involves biological systems which are themselves complicated and may also introduce complexity into the evaluation of material properties. Biological systems generally include water which may encourage an increase in particle size but may also include biosurfactants that may encourage collections of particles to disperse. Dissolved materials in biosystems may adsorb on or absorb into substances potentially affecting particle size and corresponding biological responses. For example, a solid particle that in its dry form is 50 nm may be 1000 nm in biological media or its surface may be changed so completely (such as “corona effects”) that the surface chemistry is no longer characteristic of the original MN.

The measured size of a particle is always dependent on the particular method that is being used to examine, measure or visualise the particle. Particle size is measured by using one or a number of physical phenomena whose strength depends on the size of the particle being examined. Examples of different measurement methods are diffusion velocity in liquids, electrophoretic mobility in gases and dynamic light scattering of particles or the integral surface measurement of a particle system (BET method - This method gives specific surface area of the sample and not particle size and calculations are needed to derive a particle size.). Any given particle will interact with its environment according to its own specific physical and chemical make-up. This means that the size of a particle reported by one technique may not be the same as the size when measured with another technique.

In many fields it has been the custom to define particle size ranges with common behavior, sources or composition using the method of measurement embedded in the definition. An example is the term ultrafine particles defined as particles with equivalent diameters smaller than 100 nm. Equivalent diameter refers to the practice of reporting the size of a particle of unknown composition or shape as if the particle had known composition and spherical shape. For example, when the particles are measured using an inertial based instrument, aerodynamic diameter is an equivalent diameter, computed as if the particle has unity specific gravity and a spherical shape corresponding to the measured

particle settling velocity.

Recommended Test Methods:

Sponsors may find work being performed by ISO TC229 JWG2 PG10[34] to be informative.

Particles in Air
Scanning Mobility Particle Sizer (SMPS), a combination of DMA, Dynamic Mobility Analyzer, followed by CPC.

Particles in Aqueous Media
Dynamic Light Scattering (DLS): For dilute aqueous suspensions to give hydrodynamic mean size based on cumulants analysis (z-average size and polydispersity index, see ISO 13321). Size distribution analysis (inversion) not standardised and highly subject to artifacts and data error. Before analysis by DLS, particles may be separated by centrifugation (using size of particle vs. speed of centrifuge correlations), fractionation (such as field flow), and size exclusion chromatography (using standards of appropriate shape and size) to minimise artifacts during analysis.

SEM and/or TEM: For number weighted distribution and mean size, required specific sample preparation and analysis procedures to avoid creating/counting artifacts; not appropriate for assessing frequency of small agglomerate/aggregates because of inability to differentiate between artefacts and aggregates without specific controls; also not appropriate for composite materials encapsulated in an organic surface coating. Not appropriate for some soft materials like dendrimers, polymers; best for high-z materials.

Dynamic Mobility Analyzer: For assessment of aerosol size, in conjunction with electrospray device for sampling liquid dispersion, yields number weighted aerodynamic size, not appropriate for soft materials that may collapse upon drying

Atomic Force Microscopy: For assessment of number weighted maximum profile height size of particles deposited on dry flat substrate using tapping mode, may be used in aqueous environment as well with appropriate cell; not generally appropriate for soft deformable materials, but not limited; can assess composite materials, but may not accurately report surface coatings.

Small Angle Neutron X-ray scattering: For assessment of mean size and distributed in multiple forms (dry, wet dispersed, aerosol form); best used for aqueous dispersions at moderate to low particle concentrations, appropriate for opaque suspensions not amenable to optical techniques.

Size Exclusion Chromatography: Using appropriate standards, particle size distribution may be determined for wet samples. Limitations of this technique include standards which are required and must have similar surface areas and shapes to the tested material so that they may be retained for a similar period of time in the column.

[34] ISO/TC 229/JWG2/PG10 General Framework for Determining Nanoparticle Content in Nanomaterials by Generation of Aerosols

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Term: Purity/Impurity (to be included in Composition – Pages 29 & 32)

Definition: A substance is said to be pure when its physical and chemical properties coincide with those previously established and recorded in the literature, and when no change in these properties occurs after application of the most selective fractionation techniques.

The opposite Impurity describes an unintended constituent present in a substance as produced. It may originate from the starting materials or be the result of secondary or incomplete reactions during the production process. While it is present in the final substance it was not intentionally added. It could also come from post production contamination.

Clarification: In other words, purity exists when no impurity can be detected by any experimental procedure[35].

Purity is an amount for the content of the declared substance.

Relevance to toxicology/ecotoxicology: It must be ensured that every obtained effect is based on the pure described substance and not the result of an impurity.

Impurities are then relevant, if they are present in the substance and they have toxicological and/or eco-toxicological importance. Impurities should be chemically identified, if technically possible and included in the technical specification, with stated maximum concentrations.

The scope of examinations should be dependent every time on the manufacturing process, whereas thresholds should ever depend on the manufacturing process and the toxicological properties! It is a case-by-case decision.

The manufacturer should prepare a specification with relevant impurities.

Methods often used for impurity evaluations:

- Melting point and IR-spectroscopy for powders,
- AAS and ICP-MS (AES) for metallic impurities
- UV/VIS, GC-MS^n or LC- MS^n for organic impurities

Term: Shape (Page 30)

Definition: A geometrical description of the extremities of the particle or collections of particles, agglomerates or aggregates, that make up the material under investigation. [ISO/ TC 24]

Clarification: Chemical and physical shape is determined by how the atoms in a molecule are bonded to each other and will assume the shape that most minimises electron pair repulsion. Whilst this might apply to bottom up manufacture, the shape of top down processed particles, as opposed to molecular assemblies, e.g. CNTs, will depend upon other factors, e.g. the surface tension of the liquid phase of the material if produced by laser ablation.

[35] Hawley, GG, rev., The Condensed Chemical Dictionary, Tenth Edition, Van Nostrand Reinhold Company, New York, 1981, p.871.

Relevance to toxicology/ecotoxicology: In relation to potential health effects the elongation ratio, also known as the aspect ratio may important because for those materials that have a sufficient aspect ratio such that the materials may be considered to be fibers.

Measurand: Aspect ratio, anything geology has to offer regarding "habit".

Electron microscopy could be used to describe aspect ratio.

Particle shape may be defined by a set of dimensionless terms. A number of schemes have been proposed in the past in fields as diverse as soil analysis and pharmaceuticals. The following scheme is from Heywood (1947).

If L, B and T are the length, breadth and thickness of a particle:

Elongation ratio = L/B

Flatness ratio = B/T

Sphericity = surface area of equivalent sphere/actual surface area

Circularity = circumference of circle with same area/Actual perimeter

Rugosity = actual perimeter/circumference of circumscribing circle. It is likely that shape descriptors will need to be more complex than this and it might be worth reviewing what geology/mineralogy has to offer.

Term: Solubility/Dispersibility (Pages 31-32)

Definition: Degree to which a material (the solute) can be dispersed in another material (the solvent) such that a single, temporally stable, phase results.

Clarification: The concept of solubility is relevant to both liquids and solids but not to gases. Solubility typically depends on, and increases with temperature, and might also depend on pressure. Materials that are soluble at all relative proportions are said to be completely "miscible", an example being alcohol and water, whereas materials that do not form a solution at any concentration are said to be completely insoluble or completely immiscible, an example being molybdenum and copper. Lack of solubility of one material in another is displayed by the formation of precipitates (precipitated phases) in solids and by separation into separate phases in liquids.

Note – colloidal suspensions, which might be formed by the dispersion of nanoparticles in a liquid, are not solutions but can be difficult to distinguish from solutions as they have long-term or indefinite stability. Nanoparticle detection/sizing techniques, e.g. laser scattering will be necessary to determine whether or not a sample consists of a colloidal suspension rather than a solution

From a toxicological view point, solubility in both fat and water will be important as these will affect the biological and/or environmental distribution of a material

Relevance to toxicology/ecotoxicology: If a nanomaterial is soluble in biological or environmental media then it is likely to be presented to the in-vitro/in-vivo test system in a molecular or ionic form and can be expected to elicit precisely the same response as more usual chemical forms of the material. (Note, however, that soluble materials in nanoparticle form will almost certainly dissolve more quickly than larger

forms, and that the equilibrium concentration of a solution formed from a partially soluble material in nanoparticle form might be different to that formed from larger particles of the same material.) However, if the nanomaterial under investigation is insoluble in biological or environmental media then it will be presented to the in-vitro test system in its original form and might elicit a quite different response from that expected of the chemical composition. For example:

i) Carbon nanotubes have been shown to elicit an asbestos-like response in macrophages because of their fiber morphology and biopersistence (insolubility), whereas graphite particles (of the same chemical composition, i.e. carbon, do not elicit the same response.

ii) Silicas of the group SAS, synthetic amorphous silica (comprising pyrogenic silicas, precipitated silicas, silica gels, silica sols), that may be nanostructured materials (as defined by ISO TC229 JWG1 PG6) or nano-objects (ISO TS 27687), show rather high solubility and dissolution rates in water at ambient pH and temperature. Hence high surface area nanostructured materials or nano-objects of silica will dissolve in the aqueous fluids of the biological organisms, and disappear within a few weeks from a biological organism by natural means. The subsequently formed silicates are found throughout the Earth's lithosphere (as oxygen and silicon are the most common and frequent elements in the lithosphere).

Measurand: The measurand for solubility is solubility constant – maximum mass of the solute that can be dissolved in unit mass of the solvent at standard temperature and pressure (need to check this definition).

See Media Considerations below for additional information.

Term: Stability

Definition: For nanomaterials, two types of stability must be considered: Physical stability and Chemical stability.

Physical stability (of a material): the tendency of a material to remain in its current physical state.

Chemical stability (of a material): the tendency of a material to chemically dissociate or undergo a chemical reaction in the presence of other materials.

Thermodynamic stability occurs when a system is in its lowest energy state, while kinetic stability is a measure of the time it takes for a reaction or dissociation to occur.

Clarification:

Physical stability of a nanomaterial can be affected by changes in the physical, particularly thermal, and chemical, e.g. pH, environment.

Chemical stability of a nanomaterial will be affected by changes in the physical, particularly thermal, environment. Changes in the chemical environment, e.g. pH, might also affect the chemical stability by influencing the reaction pathway.

Chemical stability is a combination of two terms - thermodynamic stability and kinetic stability.

Thermodynamic stability is a measure of the change in free energy between the starting material(s) and the product(s). The thermodynamic stability determines whether a reaction can occur.

Kinetic stability is a measure of the energy barrier that must be overcome in order for the reaction to occur.

Relevance to toxicology/ecotoxicology:

Physical stability

The state of agglomeration or aggregation will strongly affect dispersion and transport of a material, for example its ability to form a stable aerosol, e.g. affecting it penetration into the lung, or its distribution in environmental, e.g. aquatic, media. The solubility of a material might change the toxic impact of a nanomaterial, e.g., by changing its bio-distribution and/or its tendency to bio-accumulate.

Chemical stability

As chemical reactions occur at surfaces, the high specific surface area of nanomaterials means that reactions will typically propagate faster than for larger scale materials. This explains the high risk of explosion associated with nanoparticles.

In view of the potential reactivity of nanomaterials, storage under inappropriate conditions, e.g. exposure to oxygen, might adversely affect the material composition.

Measurand:

Physical Stability:

Since the physical stability will depend on the change in physical state occurring and might also depend on the chemical environment, a single measurand of physical stability does not exist. Zeta potential provides a measure of the stability of liquid suspension.

Chemical stability:

Thermodynamic stability is measured by the equilibrium constant for a particular reaction.

Kinetic stability is determined by measuring the rate of reaction, k, and calculating the energy barrier, E_a, in the Arrhenius equations:

$$k = A_o e^{-Ea/RT}$$

Stability could also be defined by a half-life time before a nanomaterial changes its phase, e.g., structural transformation or agglomerate/aggregate leading to drastic changes in the biological behavior.

See Media Considerations below for additional information.

Term: Surface Area (Page 33)

Definition: Area of the exposed surface of a particle.

Clarification: For particles, this is usually given as the specific surface area – the surface area of unit mass of the material, which for nanoparticles can easily exceed 100 m^2/g.

Can also be used to refer to the total surface area, which would include the surface area of open pores.

Relevance to toxicology/ecotoxicology: Chemical reactions take place at surfaces, hence a sample of material with a high surface area to volume ratio can be expected to have a higher reactivity than a sample of the same material with a low surface area to volume ratio.

Specific surface area appears to be relevant for a number of parameters for toxicological and ecological risk assessment. It will dictate the surface charge density in cases where nanomaterials are surface functionalised. This in turn has direct consequences on (a) nanomaterial interaction (i.e., agglomeration) with other naturally occurring particulate matter (i.e., contaminant vectors); (b) route of exposure as a function of surface ligand-biological interface (i.e., bioaccumulation pathway, bioavailability); and (c) mechanisms of toxicity (e.g., dose response curves normalised for surface area may indicate different results compared to results presented on a per mass basis).

Measurand: Specific surface area = surface area per unit mass.

NOTE: typically determined using the BET method[36] .

Term: Surface Chemistry (Page 34)

Definition: Chemical nature, including composition, of the outermost layers of the nano-object.

Clarification: Might be dominated by single atomic species, as for example in non-terminated fullerene (only carbon atoms) or inorganic fullerenes, e.g. MoS_2, where the outer atomic layer is typically sulfur, or by specific chemical moieties that have been deliberately attached to the surface for some technical reason. Note nanoparticles are often coated to reduce agglomeration and such coatings will dictate the surface chemistry of such particles.

Relevance to toxicology/ecotoxicology: Surface chemistry will affect the way nanomaterials behave in biologically relevant phenomena such as osmosis, cell function and metabolic mechanisms in plants and animals[37].

The various functionalisations of nanomaterials will lead to innumerable potential interactions and will play a key role in determining (1) fate in natural aqueous systems; (2) colloidal stability; and (3) exposure. For a given functionalisation, this in turn will affect other physico-chemical properties, such as agglomeration, dustiness, zeta potential, surface area, water solubility, etc. Thus, it is widely hypothesised and to some degree validated that surface chemistry will play one of the key roles in determining the ultimate risk of a given nanomaterial.

Measurand: Appropriate measurands might be – proportion of surface coated with different chemical species.

Potential methods:

- Zeta potential (measure of charge) – surface chemistry will affect Zeta but ZP is not a measure of surface chemistry.

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36. ISO/TC229, Nanotechnologies – Terminology and Definitions for Nanoparticles, ISO/TS 27687 ISO Copyright Office, Geneva, 2007, pp. 5.

37. Hawley, GG, rev., The Condensed Chemical Dictionary, Tenth Edition, Van Nostrand Reinhold Company, New York, 1981, p. 986.

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- Solubility as a function of pH – see above
- Thermogravimetric analysis – determine degree of functionalisation for metallic nanoparticles
- Absorption isotherm (BET) – determination of surface area
- SEM, TEM (determine surface morphology and size)
- Dynamic light scattering (determine hydrodynamic radius)

Term: Surface Charge Density (Page 33)

Definition: Electric charge present at an interface, for instance on the surface of a semiconductor material, or on the surface of a protein in water[38].

Clarification: In colloidal systems the "surface charge" can be calculated by determining the zeta potential**.** Zeta potential is an abbreviation for electrokinetic potential in colloidal systems. From a theoretical viewpoint, zeta potential is the electric potential in the interfacial double layer (DL) at the location of the slipping plane versus a point in the bulk fluid away from the interface. In other words, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle[39].

Relevance to toxicology/ecotoxicology: The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in a dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability, i.e., the solution or dispersion will resist aggregation. When the potential is low, attraction exceeds repulsion and the dispersion will break and flocculate[1]. In nanotoxicology, zeta potential (surface charge) plays a key role in determining (1) the degree of colloidal interaction which is itself a function of the pH and ionic strength of the bulk solution; and (2) bioavailability of a compound when considering mass transport through charged membranes as related to exposure.

Measurand: Zeta potential is not measurable directly but it can be calculated using theoretical models and an experimentally-determined electrophoretic mobility or dynamic electrophoretic mobility[12].

Surface charge expressed a charge density or zeta potential express in mV.

MEDIA CONSIDERATIONS

Depending on the overall approach with regard to exposure routes and exploration of the other endpoints in Annex I, the following considerations for specific media are offered.

Airborne particles

Airborne particles are typically, but not always, dry. The parameters necessary to measure airborne nanomaterials are particle number per unit of volume, median and distribution of mass, and aerodynamic diameter. Particle number, using a condensation particle counter (CPC), is valuable data and relatively simple to obtain. These are light scattering devices that assume a spherical particle, which is not always the case, e.g., CNTs. Median diameter (aerodynamic) and distribution are essential, but these are not straightforward to measure for nanomaterials. The 'gold standard' for aerodynamic diameter of aerosols is cascade impaction, and there are impactors that have cutoff sizes in the nano-range. Typically, these need a

38. http://en.wikipedia.org/wiki/Surface_charge
39. McNeil, S., "Terms Used at NCI (National Cancer Institute)-NCL (Nanotech Characterisation Lab)," Frederick, MD, 2008.

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lot of air to pass through before the mass can be measured with any accuracy; that limits their utility because nanomaterials simply don't weight very much. None-the-less, an impactor can indicate if there are agglomerates present, which is important. It is also worthwhile to have the filter or slide samples examined under a microscope (EM, for example) to see if the particles measured are really nanomaterials. An SMPS is the typical instrument for size distribution of nanomaterials, but it is not very mobile and is not a common 'lab' piece of equipment. For all these instruments, calibration is key – and is often challenging.

All these need to be measured while the nanomaterial is in the air, because experience has taught that generation methods may alter the characteristics of the particle.

Particles in Aqueous Suspension

Circumstances where particles exist in an aqueous suspension include certain physical-chemical analysis, contact with an organism (internally or externally) or in an environmental medium.

Water samples

Investigators are asked to consider examining aqueous based physical-chemical properties in the following aqueous solutions:

- Medium hardness at pH 7 at 3 concentrations (e.g. 10, 1 and 0.1 mg/L for more dispersal materials)
- Medium hardness with pH at 4, 7 and 9
- Low, medium and hard water at pH 7,
- pH 7 with a low, medium and high well described NOM isolate (e.g. Standard Suwannee River Humic Acid (SRHA) @ 1, 5, 10ppm)
- Standardised sea water
- Iso-tonic solution (0.9% w/v NaCl)
- Artificial saliva
- Artificial serum

It is recommended that investigators first examine the particular property using 3 concentrations under consistent conditions. Results of this analysis will inform on the most relevant concentration to be examined in further testing. For all hardnesses, the concentration of all the major ions should be described as well as the specific ionic strength. In addition, pH adjustments should be ideally undertaken with NaOH/HCl, where possible. Natural Organic Matter (NOM) isolate should be well described and taken by reverse osmosis. Ideally a standardised NOM should be used such as SRHA. If ideal test conditions cannot be achieved, then explanations should be provided.

Testing of properties is encouraged under these various media in order that we may better understand the material's behavior under a variety of conditions. Furthermore, investigators are invited to generate data in additional media based on trends observed in results or specific experience or insight.

Preparation and Analysis

Before undertaking testing with nano particles, loosely bound agglomerates should be dispersed using slow agitation.

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ANNEX III.
DATA SHARING TEMPLATE FORMAT[40]

Introduction

This document is intended to provide guidance to lead sponsors, co-sponsors and other contributors of the OECD Sponsorship Programme of Testing a Representative Set of Manufactured Nanomaterials on how to report their results within the programme. This document is composed of three parts:

- The **first part** describes essential elements to be included in the dossiers in order to facilitate data sharing and comparison as well as essential elements to be included in the templates when reporting results for the different endpoints/characteristics;
- The **second part** contains two examples of robust study template taken from the Screening Information DataSets (SIDS) Dossiers Guidance[41]: i) acute toxicity to fish; ii) mammalian toxicity (repeated dose toxicity). These examples, as well as all the templates in the SIDS guidance, should be further developed with regard to the amount of details that the templates prompt for, and thus there is a need to review and expand the templates to address all the points stated in the first part. Eventually this second part should be expanded to contain the actual updated reporting templates for all endpoints; and
- The **third part** gives guidance on templates for non-standardised test methods.

Data sharing

The format of templates used for sharing study results should be standardised to ensure that all relevant information is captured and make it easier for the reader to go through the information. The format should include a thorough description of the material tested, provide a detailed enough study description to enable a clear understanding of how the study was performed and describe the results in detail, so to allow a comparison with other studies and materials.

Learning from the OECD HPV program as well as other past experiences of reporting test results within several programmes for different kinds of chemicals (e.g. the HPVs, pesticides, biocides and new chemicals), and from REACH, which also integrated these experiences, an appropriate approach seems to be data sharing in form of robust study summaries. The robustness, i.e. the elaboration and recording of all relevant information in detail, must be underlined as exactly this allows the scientific acceptance of the summary studies between stakeholders and between programmes.

Within the European Union an in-depth discussion on robust study summaries has taken place, leading to the integration of the experience from the industrial chemicals, the pesticides and biocides legislation, in agreed formats for submitting robust study summaries for industrial chemicals and biocides to the authorities. These formats have been implemented as a database, namely the recently developed version 5 of IUCLID. This reporting format might seem complex, however, it resembles directly what a potential registrant would need to provide to the regulatory authorities and, in addition, has the advantage of exploiting IUCLID that is, as well, an accepted tool for data information exchange within the OECD.

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[40] The Annex III has added in April 2010 as agreed by the WPMN at its 6th meeting.

41 . http://www.oecd.org/dataoecd/13/17/36045066.pdf

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In the following, a (possibly) comprehensive list of entries that could be included in such a format is given. Some of these may not be relevant for all nanomaterials, so they might be simply labelled as not applicable in the report on the individual nanomaterial. It is important to keep the order of appearance given, to ensure a direct compatibility with IUCLID.

On this basis, it would then be possible to generate templates similar to the ones of the OECD SIDS Dossiers, which are compiled in the "Guidance for Completing a SIDS Dossier"[42].

Those templates were generated for communicating test results obtained for industrial chemicals and seem to be an excellent starting point for discussing how to best achieve data sharing of studies with nanomaterials. For nanomaterials it is recommended that many fields are extended, e.g. the description of the test-material, including its stability, homogeneity and influence of the media/matrix/vehicle, in order to ensure that the data are really comparable between different studies and materials.

PART I: ESSENTIAL ELEMENTS FOR THE DOSSIERS AND TEMPLATES

The OECD Sponsorship Programme of Testing a Representative Set of Manufactured Nanomaterials on intends to develop dossiers on selected Nanomaterials. In order to help in extracting relevant information from this programme, it is opportune to set a common general structure for these dossiers, which will allow easy extraction of the information as well as comparison among different nanomaterials. On the other hand, this template should serve as a guide for contributors on how to report their results within the programme. According to the *Guidance Manual for the Testing of Manufactured Nanomaterials: OECD's Sponsorship Programme*, the following information should be reported for all tested materials and studies (as appropriate):

I.1 Nanomaterial Information/Identification

The information given should be sufficient to enable each substance to be identified. If it is not technically possible or if it does not appear scientifically necessary to give information on one or more items below, the reason should be clearly stated.

- Nanomaterial name (chemical name (e.g. IUPAC) and, if different, name from list).
- CAS Number.
- EC number (if available).
- Structural formula/molecular structure.
- Composition of nanomaterial being tested, including degree of purity in %, nature of known impurities including isomers and by-products (in % or ppm) or additive(s) (in ppm or %) (e.g. stabilising agents, surfactants or inhibitors. As appropriate.
- Spectral data (ultra-violet, infra-red, nuclear magnetic resonance or mass spectrum) (If appropriate).

[42] See http://www.oecd.org/dataoecd/13/17/36045066.pdf

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- High performance liquid chromatogram, gas chromatogram (If appropriate).
- Description of the analytical methods or the appropriate bibliographical references for the identification of the material and, where appropriate, for the identification of impurities and additives. This information should be sufficient to allow the methods to be reproduced.
- Basic morphology
- Description of surface chemistry (e.g., coating or modification)
- Major commercial uses
- Known catalytic activity
- Method of production (e.g., precipitation, gas phase)
- Producer/provider
- Batch no, and any other information useful to univocally identify the material used

The following properties and characteristics should be reported for all materials and studies (as appropriate).

I.2 Physical-Chemical Properties and Material Characterisation

The following list is a list of physical-chemical properties that might be relevant to address for nanomaterials, but for the individual nanomaterials an evaluation of the relevance of each property should take place taking into account also stated limits of the applicability of the different tests methods, and the nature of the tested material.

Characterisation of the material as on the shelf:

- Appearance, (if applicable).
- Melting point, (if applicable).
- Density, (if applicable).
- Particle size including size distribution
- n-octanol-water partition coefficient, where relevant
- Water solubility/dispersibility, hydrophilicity
- Solubility/dispersability in organic solvents, oleophilicity
- Auto flammability, (if applicable).
- Flammability, (if applicable).
- Explosiveness, (if applicable).

- Oxidising properties, (if applicable).
- Oxidation reduction potential
- Stability in organic solvents and identity of relevant degradation products
- Storage stability and reactivity towards container material
- Stability; thermal, sunlight, metals
- pH, (if applicable).
- Dissociation constant, (if applicable).
- Other additional relevant physico-chemical information,(where available)
- Agglomeration/aggregation
- Crystalline phase
- Crystallite and grain size
- Aspect ratio, shape, (from e.g. TEM, SEM)
- Specific surface area
- Zeta potential (surface charge)
- Surface chemistry (where appropriate)
- Stability and homogeneity (on the shelf, in water/organic solvents)
- Dustiness
- Porosity, pore density
- Photocatalytic activity
- Pour density
- Radical formation potential
- Catalytic activity

Characterisation, homogeneity and stability of prepared test items[43]*in respective media (as appropriate):*

- Test item preparation protocol, conditioning (for test media, test matrices as used for toxicological assessment and fate analysis), homogeneity (minimum sample intake), short term stability
- Physical chemical properties:
  - composition/purity
  - size and size distribution
  - agglomeration/ aggregation
- Zeta-Potential
- Biophysical properties (as appropriate):
  - protein binding/corona characterisation
  - residence times
  - adsorption enthalpy
  - conformation changes on binding

I.3 Endpoints

The following endpoints should be addressed for all nanomaterials as appropriate.

Environmental Fate and behaviour

- Stability
  - Phototransformation in air
  - Hydrolysis
  - Phototransformation in water
  - Dispersion stability in water
  - Abiotic degradability and fate (other)
- Biodegradation (as appropriate, depending on the material).
  - Biodegradation in water: screening tests

[43] This is intended to assess how sample preparation procedures and media may influence the properties and behaviour of the nanomaterials and the results of subsequent testing.

  - Biodegradation in water and sediment: simulation tests
  - Biodegradation in soil
  - Identification of degradation product(s)
  - Sewage treatment simulation testing
- Bioaccumulation (as appropriate, depending on the material).
  - Bioaccumulation: aquatic/sediment
  - Bioaccumulation: terrestrial
- Transport and distribution
  - Adsorption/desorption
  - Other distribution data
- Other relevant information (when available)

Environmental Toxicity

- Aquatic toxicity
  - Short-term toxicity to fish
  - Long term toxicity to fish
  - Short term toxicity to aquatic invertebrates
  - Long term toxicity to aquatic invertebrates
  - Toxicity to aquatic algae and cyanobacteria
  - Sediment toxicity
  - Terrestrial toxicity
- Toxicity of soil macroorganisms except arthropods
- Toxicity to terrestrial arthropods
  - Toxicity to soil microorganisms
- Additional ecotoxicological information

Mammalian Toxicity

(As appropriate)

- Pharmacokinetics, ADME
  - Basic toxicokinetics
  - Dermal absorption
- Acute toxicity
  - Acute toxicity: oral
  - Acute toxicity: inhalation
  - Acute toxicity: dermal
  - Acute toxicity: other routes
- Irritation/corrosion
  - Skin irritation/corrosion
  - Eye irritation
- Sensitisation
  - Skin sensitisation
- Repeated dose toxicity
  - Repeated dose toxicity: oral
  - Repeated dose toxicity: inhalation
  - Repeated dose toxicity: dermal
- Genetic toxicity
  - Genetic toxicity *in vitro*
  - Genetic toxicity *in vivo*
- Toxicity to reproduction
  - Toxicity to reproduction
  - Developmental toxicity/teratogenicity
  - Toxicity to reproduction: other tests
- Specific investigations:
  - Neurotoxicity

- Immunotoxicity
- Other Specific Investigations
  - Phototoxicity
  - Blood compatibility/ blood toxicity
  - Cardiovascular toxicity
- Other test data
- Chronic Toxicity
- Exposure related to humans

I.4 Material Safety

To ensure appropriate and safe handling of the material, information on the following properties should be investigated and adequately addressed:

- Flammability
- Explosivity
- Incompatibility

I.5 Appearance and organisation of the study reports

General appearance of the templates

Within each study, the following sections should be included:

- Test material identification (on the shelf, as before)
- Test materials details, test item preparation, conditioning protocols, characterisation and stability (free text describing any relevant details)
- Methods
- Test conditions
- Results
- Discussion/Remarks
- Conclusion
- References

Test material section

The following details should be given in the test material sections:

- Nanomaterial name
- CAS number
- EC number, if available
- Structural formula / molecular structure
- Composition of the nanomaterial being tested (including purity, impurities or additives)
- Characteristics / Identifiers specific to nanomaterials
  - Characterisation, homogeneity and (short term) stability of prepared test items:
    - Test item preparation protocol, conditioning (for test media, test matrices as used for toxicological assessment and fate analysis), homogeneity (minimum sample intake), short term stability
    - Physical chemical properties:
      - composition/purity
      - size and size distribution
      - agglomeration/ aggregation
      - Zeta-Potential
    - Biophysical properties:
      - corona characterisation
      - residence times
      - adsorption enthalpy
      - conformation changes on binding
    - Other relevant information

Methods section

The following details should be given in the methods sections:

- Test guideline
- Year of the test guideline

- Modifications of the guideline
- Species / Strain
- Exposure route
- Exposure duration
- GLP
- Analytics (in particular, analytical verification of doses or concentrations)
- Statistics
- Any other relevant information

Test conditions section

The following details should be given in the test conditions section:

- Description of the test item preparation protocol
- Description of the test item
- Description of the test vehicle/media/matrix
- Homogeneity and stability in test media and conditions

Results section

The following details should be given in the in results section:

- According to the guidance on SIDS dossiers a detailed description of all relevant findings

Discussion/remarks section

The following details should be given in the in discussion/remarks section:

- This section should describe how the results were used and interpreted to reach the conclusions. In particular, it should address whether and how any conclusion on "classification or non-classification" for the addressed endpoint can be derived from this particular study results.

Conclusions section

The following details should be given in the in the conclusions section:

- The study authors conclusion as well as the sponsor's conclusion are stated

Reference section

The following details should be given in the in the reference section:

- Identity of testing laboratory
- Reference to study number,
- Report number
- Report date

The information given above is illustrated in part two of this document where some examples of how the information is collected and presented are given.

PART II: EXAMPLES

These examples, focusing on a few end-points: acute toxicity to fish and repeated dose toxicity, have been reviewed and expanded to address the details and points stated in Part I and its structure aligned with it. The remaining the templates in the SIDS guidance would also need to be similarly modified.

Example: Acute toxicity to fish

In the following, a **draft** template for reporting the outcome of the study on acute toxicity to fish, which was taken from the Screening Information DataSets (SIDS) Dossiers Guidance and adapted for this programme is shown:

Test Substance
Identity (purity):
Nanomaterial name:
CAS number:
EC number, if available
Structural formula / molecular structure:
Composition of the nanomaterial being tested (including purity, impurities or additives):

Characteristics / Identifiers specific to nanomaterials:
Characterisation, homogeneity and (short term) stability of prepared test items:
Test item preparation protocol, conditioning (for test media, test matrices as used for toxicological assessment and fate analysis), homogeneity (minimum sample intake), short term stability
Physical chemical properties:
composition/purity
size and size distribution
agglomeration/ aggregation
Zeta-Potential
Biophysical properties:
corona characterisation
residence times
adsorption enthalpy
conformation changes on binding
Other relevant information

Method
Method/guideline followed *(experimental/calculated)*:
Year of the Test Guideline version:

Test type *(static, semi-static, flow-through, field observation):*
Year *(study performed):*
GLP: Yes [ ] No [ ]
Species/Strain/Supplier:
Analytical Monitoring:
Exposure period*[Duration]:*
Doses/concentration levels:
Frequency of treatment:
Control group and treatment:
Post exposure observation period:
Statistical methods:
Other relevant information:

Test Conditions: (*Detail and discuss any significant protocol deviations, and detail differences from the guideline followed including the following as appropriate:*

- *Test Item*
  - *Description of the test item preparation protocol*
  - *Description of the test item*
  - *Description of the test vehicle/media/matrix*
  - *Homogeneity and stability in test media and conditions*

- *Test fish (Age/length/weight, loading, pretreatment):*

- *Test conditions, for example:*
  - *Dilution water source:*
  - *Dilution water chemistry (hardness, alkalinity, pH, TOC, TSS, salinity):*
  - *Stock and test solution and how they are prepared:*
  - *Concentrations dosing rate, flow-through rate, in what medium:*
  - *Vehicle/solvent and concentrations:*
  - *Stability of the test chemical solutions:*
  - *Exposure vessel type (e.g., size, headspace, sealed, aeration, lighting, # per treatment):*
  - *Number of replicates, fish per replicate:*
  - *Water chemistry in test (D.O., pH) in the control and one concentration where effects were observed:*

- *Test temperature range:*
- *Method of calculating mean measured concentrations (i.e. arithmetic mean, geometric mean, etc.) :*

Results

Nominal concentrations *(as mg/L):*
Measured concentrations *(as mg/L):*
Unit *(results expressed in what unit):*
Element value *(e.g. LC_{50}, LC_o, LL_{50}, or LL_0 at 48, 72 and 96 hours, etc., based on measured or nominal: concentrations):*
Statistical results *(as appropriate*):
Effect concentration instead of element value (or appropriate metrics)

Remarks **(***Discuss if the effect concentration is greater than the solubility of the substance in the test medium. Describe additional information that may be needed to adequately assess data for reliability and use, including the following:*

- *Biological observations:*
- *Table showing cumulative mortality:*
- *Lowest test substance concentration causing 100% mortality:*
- *Mortality of controls:*
- *Abnormal responses:*
- *Reference substances (if used) – results:*
- *Any observations, such as precipitation that might cause a difference between measured and nominal values:*
- *length of (and effects observed during) post-exposure observation period*
- *basis for effect, if known*

Conclusions

Discussion/Remarks on the conclusions: *(Describe how the results were used and interpreted to reach the conclusions. In particular, it should address whether and how any conclusion on "classification or non-classification" for the addressed endpoint can be derived from this particular study results.* Remarks: *(Identify source of comment)*

Reliability

(Data reliability code, e.g. Klimisch code, if used, possibly a flag for 'key study')

Remarks: *(The rationale for the reliability code should be described clearly as should the process by which the "Reliability" decision was made)*

References *(Free Text) including:*

Identity of testing laboratory
Reference to study number,
Report number
Report date

Other

Last changed: *(administrative field for updating)*
Order number for sorting: *(administrative field)*

Remarks *(Use for any other comments necessary for clarification.)*

In the following, a **draft** template for reporting the outcome of the study on acute toxicity to fish, which was taken from the Screening Information DataSets (SIDS) Dossiers Guidance and adapted for this programme is shown:

Example: Mammalian Toxicity: Repeated Dose Toxicity

Test Substance
Identity (purity):
Nanomaterial name:
CAS number:
EC number, if available
Structural formula / molecular structure:
Composition of the nanomaterial being tested (including purity, impurities or additives):
Characteristics / Identifiers specific to nanomaterials:
Characterisation, homogeneity and (short term) stability of prepared test items:
Test item preparation protocol, conditioning (for test media, test matrices as used for toxicological assessment and fate analysis), homogeneity (minimum sample intake), short term stability
Physical chemical properties:
composition/purity
size and size distribution
agglomeration/ aggregation
Zeta-Potential
Biophysical properties:
corona characterisation
residence times
adsorption enthalpy
conformation changes on binding
Other relevant information

Method
Method/guideline followed:
Year of the Test Guideline version:
Modifications/deviations of the test guideline (if any):
Test type:
Year (*study performed*):
Species:
Strain:
Route of administration, oral (gavage, drinking water, and feed), dermal, inhalation (aerosol, vapour, gas, particulate), other:
Exposure period:
GLP: Yes [ ] No [ ]
Duration of test:
Doses/concentration levels:
Sex:
Frequency of treatment:
Control group and treatment:
Post exposure observation period:
Statistical methods:
Other relevant information:

Test Conditions (*Detail and discuss any significant protocol deviations and detail differences from the guideline followed including the following as appropriate):*

Test Item
Description of the test item preparation protocol
Description of the test item
Description of the test vehicle/media/matrix
Homogeneity and stability in test media and conditions

- *Test Subjects*

  - *Age at study initiation:*
  - *No. of animals per sex per dose:*
- *Study Design*

  - *Vehicle:*
  - *Satellite groups and reasons they were added:*
  - *Data on positive controls (if used)*
  - *Clinical observations performed and frequency (clinical pathology, functional observations, etc.):*
  - *Organs examined at necropsy (macroscopic and microscopic):*

Results

NOAEL (NOEL):
LOAEL (LOEL):
Actual dose received by dose level by sex (if known):
Toxic response/effects by dose level:
Statistical results (as appropriate)

Discussion/Remarks on the results: *(Describe additional information that may be needed to adequately assess data for reliability and use, including the following if available. Provide at a minimum qualitative descriptions of elements where dose effect related observations were seen):*

- *Body weight:*
- *Food/water consumption:*
- *Description, severity, time of onset and duration of clinical signs:*
- *Ophthalmologic findings incidence and severity:*
- *Haematological findings incidence and severity:*
- *Clinical biochemistry (including urinanalysis) findings incidence and severity:*
- *Mortality and time to death:*
- *Gross pathology incidence and severity:*
- *Organ weight changes:*
- *Histopathology incidence and severity* :
- *Neurobehaviour observations (if appropriate)*

Conclusions

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Discussion/Remarks on the conclusions: *(Describe how the results were used and interpreted to reach the conclusions. In particular, it should address whether and how any conclusion on "classification or non-classification" for the addressed endpoint can be derived from this particular study results.* Remarks: *(Identify source of comment)*

Reliability
(Data reliability code, e.g. Klimisch code, if used, possibly a flag for 'key study')

Remarks: *(The rationale for the reliability code should be described clearly as should the process by which the "Reliability" decision was made)*

References *(Free Text,) including:*
Identity of testing laboratory
Reference to study number,
Report number
Report date

Other

Last changed: *(administrative field for updating)*
Order number for sorting: *(administrative field)*

Remarks *(Use for any other comments necessary for clarification.)*

PART III: DRAFT PROPOSAL FOR DATA SHARING FORMAT FOR NON-STANDARDISED TEST METHODS

The information collected in the template for non-standardised methods should be, as far as possible, in line with the information required for the standardised methods.

Test substance identification:

(as far as possible, as in part I)

- Nanomaterial name (chemical name (e.g. IUPAC) and, if different, name from list)
- CAS Number
- Structural formula/molecular structure.
- Composition of nanomaterial being tested, including degree of purity in %, nature of known impurities including isomers and by-products (in % or ppm) or additive(s) (in ppm or %) (e.g. stabilising agents, surfactants or inhibitors. As appropriate.
- Basic morphology
- Description of surface chemistry (e.g., coating or modification)
- Major commercial uses
- Known catalytic activity
- Method of production (e.g., precipitation, gas phase)
- Producer/provider

- *Batch no, and any other information* useful to univocally identify the material used
- Other relevant information

Test substance characterisation[44]:

Research Field

- Characterisation
- Physico-chemical properties
- Environmental Fate
- Ecotoxicology
- Toxicology
- Exposure Assessment Environment (including environmental safety)
- Exposure Assessment Human (including workplace safety)

Study result type:

- Experimental result
- Estimated by calculation
- Read-across
- QSAR
- Other

Method

- *In vitro*
  - Test system:
- *In vivo*
  - Species:
- Sample administration
- Exposure route
- Exposure duration
- Description of the method and give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy, or other.
- Analytics (in particular, analytical verification of doses or concentrations)
- Statistics
- Any other relevant information

Test conditions[45]:

- Description of the test item preparation protocol
- Description of the test item

[44] As far as possible, as in part I and, if available, both for the material as "on the shelf" and for the prepared test item.

[45] See also Part I (page 66-75).

- Description of the test vehicle/media/matrix
- Homogeneity and stability in test media and conditions

Reliability:

- Quality control: Indicate whether GLP was applied or other quality control measures.
- Minimum performance criteria

Results:

Summary of all the relevant results according, as far as possible, to the guidance on SIDS dossiers

Discussion/Remarks:

Description of how the results were used and interpreted to reach the conclusions. In particular, it should address whether and how any conclusion on "classification or non-classification" for the addressed endpoint can be derived from this particular study results.

Conclusion:

The study author's conclusion, as well as the sponsor's conclusion should be stated.

Reference:

Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages). Company, if appropriate.

Useful links

OECD Guidance on Completing a SIDS Dossier

http://www.oecd.org/dataoecd/13/17/36045066.pdf

ECHA Guidance for identification and naming of substances under REACH

http://reach.jrc.it/docs/guidance_document/substance_id_en.pdf

ECHA Guidance on data-sharing

http://reach.jrc.it/docs/guidance_document/data_sharing_en.pdf

ECHA Guidance on substance-evaluation

http://reach.jrc.it/docs/guidance_document/evaluation_en.pdf

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ANNEX IV.
ALTERNATIVE METHODS IN THE SPONSORSHIP PROGRAMME

1. Alternative methods are an integral part of the WPMN's work on the Sponsorship Programme. This will be particularly relevant as the work of the WPMN steering group on The Role of Alternative Methods in Nanotoxicology (SG7) evolves. It is foreseen that this section will be developed in parallel with WNT-programme activities on the validation of the test guidelines and form a part of the development of an overall strategy for reducing the use of animals in the hazard evaluation of nanomaterials and for the identification of unforeseen toxicity concerns or biological mechanisms of action.

2. An alternative method is a test that: (i) reduces the number of animals required; (ii) refines procedures to lessen or eliminate pain or distress to animals, or enhances animal well-being; (iii) or replaces animals with non-animal systems or with less sentient species. This refers directly to the 3Rs principle of refinement, reduction and replacement, which should be respected in the sponsorship programme. Modern toxicology makes use of integrated testing strategies (ITS). This includes application of a weight of evidence (WoE) approach for certain toxicological endpoints or information requirements. This WoE takes into account all available sources of information and types or formats of data.

In the sponsorship programme the application of the 3Rs is addressed on three different levels for an endpoint and across endpoints:

- Existing information is used in a WoE approach, comprising as well physico-chemical information and data derived by using *in silico* models (also referred to as "non-test methods") as far as applicable and existing.

- New data needs to be generated for MN, if no reliable and adequate data exist. *In vitro* methods may be used to generate new information related to the agreed upon endpoints in the program requirements when standardized test methods exist. Other assays that address mode of action may be considered by the sponsors in parallel with the *in vivo* assays, such that new biomarkers of effect and/or of susceptibility might be reliably identified *in vitro* and subsequently verified and recognised to be predictive for a relevant adverse effect. There are *in vitro* methods for some OECD health effects guidelines e.g. genotoxicity and skin absorption and these are an integral part of testing strategies for the hazard assessment of chemicals. Similarly, for this sponsorship programme, sponsors should seriously consider relevant *in vitro* methods in the development of the Dossier Developmental Plans (DDPs). Potential assays that the sponsors may consider in addition to *in vitro* methods in OECD guidelines are described in the tables Y and Z (see Annexes).

- If existing information collected from all available sources and data generated are deemed to be insufficient for a given endpoint, *in vivo* methods are used. Testing strategies applied should aim at using minimum animal numbers for maximum information outcome. This concerns as well combination of studies for several endpoints. Thus, sponsors should strive for developing testing strategies that include *in vitro* methods.

Selection and prioritisation of test methods

3. At this stage, it is not yet possible to make recommendations for specific alternative approaches to be used in testing nanomaterials. However, in order to be consistent with the flexibility of phase 1 of the

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Sponsorship Programme sponsors may consider evaluating in parallel how information (including identification of unforeseen toxicity concerns and knowledge of biological mechanisms of action) generated through alternative methods can be used.

4. Some methods, though not all inclusive, that have been used and reported in the literature (including some assays that need to be standardized and validated) have been collated and sorted by endpoints and corresponding effects that these methods may display.

5. These tests are summarized in Table Z. They were analysed and sorted according to endpoints as addressed in the sponsorship programme, and further nano-specific needs so far identified. Endpoints and sub-endpoints or effects are addressed, which will support subsequent use of information generated in targeted weight of evidence approaches and in support to the development of testing strategies as addressed below.

6. Table Z of test methods may serve as a resource for selection and prioritisation. The table also highlights possible interferences with assays, which may not offer reliability or relevance for a specific purpose if applied improperly. Further collaboration in the frame of SG7 foresees to collate corresponding references and possible protocols for use related to identified and prioritised methods to further develop table Z.

Based on scientific evidence, suitable candidate assays are prioritized in five sequential steps:

i) Compare purpose, intended use and method description with nano-specific needs (sub-endpoints) and address the areas; update as well with SG4 review on nano-specific concerns and corresponding information requirements.

ii) Identify NON reliable tests/measurement parameters (e.g. interferences of MN with test system components) and attribute low priority and/or describe pitfalls.

iii) Identify NON relevant tests/measurement parameters (e.g. test system components or cells used that are not representative) and attribute low priority and/or describe pitfalls.

iv) Selection of key assays for use in the Sponsorship Programme taking into account indicated links from SG4, systems toxicology, and priority items identified from nano-specific information needs. RELEVANCE.

v) Set order of priority according to i) to iv) and thereafter set order of priority according to the feasibility of test method standardization, transferability and overall RELIABILITY.

Validation of test methods

7. A large number of *in vitro* assays are currently used, but the suitability and validity remains unclear as reference results are missing. In accordance with the needs of the present programme, criteria are presented which shall support selection of test methods for relevant endpoints and enable method prioritisation. These criteria are directly derived from the OECD Guidance Document no 34 and support the scientific validation process with due regard to the test guidelines in WNT.

8. The test method validation process targets two elements: Reliability (repeatability/ reproducibility) and relevance (predictability). A specific issue for nano-related endpoints is the lack of existing data. For MNs scientific acceptance criteria related to the validation of test methods are not available, because they have not yet been established. Such criteria would be described according to OECD

Guidance document 34[46]. Test method definition and information according to this guidance document should be collected and developed with due regard to formal validation of the test method used.

Quality assurance and test item preparation

9. Even if testing in the frame of the sponsorship programme is not performed under GLP it should be based on its principles. This comprises the need to use a well defined test item and vehicle/matrix/medium. Test item preparation has been identified as a most critical step. Test item properties have to be addressed along with physico-chemical properties following sample preparation. For a specific test a suitable test item related set of information has to be developed. Information on other properties, such as corona formation (for example, surface modifications by proteins, lipids, solvents and salts) may be pivotal depending on the vehicles/matrices/media used.

Testing strategies

10. A long-term vision and strategy for toxicity testing in the future might eliminate many of the *in vivo* assays currently used. A testing strategy will be described consisting of tiered test schemes and/or test batteries, that are expected to be more predictive of the *in vivo* response than could be obtained from any individual component of the testing system. Just like an individual toxicity test, a test scheme and/or test battery can be validated for use in regulatory decision making following the principles outlined in [ENV/JM/MONO(2005)14]. Integrated testing strategies are understood as approaches that integrate different types of data and information into the decision-making process. In addition to the information from individual assays, test batteries, and/or tiered test schemes, integrated testing strategies may incorporate approaches such as weight-of-evidence and exposure/population data into the final risk assessment for a substance.

11. In summary, testing strategies used in this programme should aim at using minimum animal numbers for maximum information outcome with respect to making predictions. This concerns as well combination of studies for several endpoints.

12. Sponsors may consider tests which provide mechanistic information and information on perturbations of metabolic pathways based on the findings from *in vivo* test methods. Modern toxicology not only addresses apical/gross toxicity endpoints, but may use systems toxicology platforms and methodology including high throughput assay technology to characterise biological responses of certain cell or tissue models upon exposure to chemicals.

[46] Guidance document on the validation and international acceptance of new or updated test methods for hazard assessment, Series on Testing and Assessment No. 34, OECD Environment Directorate, Paris, 18 August 2005 (2005)

Table Y

Validated *In Vitro* Tests for Chemicals
Genotoxicity Assays OECD TG 471 Bacterial Reverse mutation assay OECD TG 473 *In Vitro* mammalian chromosome aberration test OECD TG 476 *In Vitro* mammalian cell gene mutation test Skin corrosivity tests OECD TG 430 *In vitro* skin corrosion test: transcutaneous electrical resistance test OECD TG 431 *In vitro* skin corrosion test: human skin model test OECD TG 435 *In vitro* membrane barrier test for skin corrosion Phototoxicity OECD TG 432 *In vitro* 3T3 NRU phototoxicity test Skin absorption OECD TG 428 skin absorption *in vitro* test.
Severe eye irritation and corrosion OECD TG 437. The Bovine Corneal Opacity and Permeability (BCOP) Test Method for Identifying Ocular Corrosives and Severe Irritants. OECD TG 438. The Isolated Chicken Eye (ICE) Test Method for Identifying Ocular Corrosives and Severe Irritants
Estrogenic Agonist-Activity of Chemicals TG 455. Stably Transfected Human Estrogen Receptor-a Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals.
Near - OECD validated assays, accepted by EC
Skin irritation In Vitro Skin Irritation: Reconstructed Human Epidermis (RhE) Test Method Under evaluation by OECD WNT; accepted in the EU: Test Method Regulation (EC) B46
ECVAM – ESAC endorsed validation
Embryotoxicity/teratogenicity Embryonic stem cell test for embryotoxicity.

Table Z: Summary of available vitro test systems sorted by (sub-) endpoints

Endpoint	*In vitro* model (standardized cell lines/primary cells)	Measurement	Comments
Cytotoxicity (dose-effect relationship)			
Cells and cell layer morphology	Adherent cells (HaCaT, CaCo-2, CaLu3, RLE-6NT, A549, NRK-52E, MDCK, HepG2, Balb/3T3, NIH 3T3)	Phase contrast microscopy	MN precipitations may affect cell detection.
		Digital Holography for cell morphology alterations	No known MN effects.
Epithelial barrier integrity	Epithelial cells with intact tight junctions (HaCaT, CaCo-2, CaLu3, RLE-6NT, NRK-52E, MDCK, HepG2)	Trans Epithelial Electrical Resistance (TEER) = Impedance sensing.	No known MN effects.
		Optical methods e.g. Digital Holography for cell morphology alterations	No known MN effects.
Cell viability, cellular metabolic activity	Adherent cells: HaCaT, CaCo-2, CaLu3, RLE-6NT, A549, NRK-52E, MDCK, HepG2, Balb/3T3, NIH 3T3 Suspension cells: JURKAT, MonoMac, U937, THP-1, RAW-294.7	LDH release	MN may adsorb LDH or LDH substrates or interfere with colour detection.
		Neutral Red uptake	MN may adsorb Neutral Red or interfere with colour detection.
		Propidium Iodide uptake	MN may adsorb PI or interfere with fluorescence detection.
		Tetrazolium salts MTT, XTT, WST-1, MTS assays	MN may adsorb Tetrazolium salts or interfere with colour detection.
		Alamar blue assay	MN may adsorb Alamar blue or interfere with colour detection.
		BrdU assay	No known MN effects.
Proliferation	Adherent cells: HaCaT, CaCo-2, CaLu3, RLE-6NT, A549, NRK-52E, MDCK, HepG2, Balb/3T3, NIH 3T3	Tetrazolium salts MTT, XTT, WST-1, MTS assays	MN may adsorb Tetrazolium salts or interfere with colour detection.
		Alamar blue assay	MN may adsorb Alamar blue or interfere with colour detection.

	Suspension cells: JURKAT, MonoMac, U937, THP-1, RAW-294.7	BrdU assay	No known MN effects.
		Cell counting (FACS, Casy)	No known MN effects.
		Trans Epithelial Electrical Resistance (TEER) = Impedance sensing.	No known MN effects.
Cell death: necrosis, apoptosis (membrane integrity, induction of apoptotic cascade)	Adherent cells: HaCaT, CaCo-2, CaLu3, RLE-6NT, A549, NRK-52E, MDCK, HepG2, Balb/3T3, NIH 3T3 Suspension cells: JURKAT, MonoMac, U937, THP-1, RAW-294.7	Caspase-3 activation	No known MN effects.
		Annexin V binding	MN may adsorb Annexin V or interfere with fluorescence detection.
		bcl2/bax ratio by ELISA	No known MN effects.
		LDH release	MN may adsorb LDH or LDH substrates or interfere with colour detection.
		Propidium Iodide uptake	MN may adsorb PI or interfere with fluorescence detection.
		Cell morphology alterations detected by optical methods (Digital Holography) or impedance sensing	No known MN effects.
Stress response			
Oxidative stress	Adherent cells: HaCaT, CaCo-2, CaLu3, RLE-6NT, A549, NRK-52E, MDCK, HepG2, Balb/3T3, NIH 3T3 Suspension cells: JURKAT, MonoMac, U937, THP-1, RAW-294.7 endothelial cells, PBMC	ROS assay (e.g. H_2DCF-DA)	MN may adsorb DCF-DA or interfere with fluorescence detection.
		Modified comet assay (oxidative DNA damage)	No known MN effects. Time intensive.
		GSH depletion assay	No known MN effects.
		SOD assay	MN may adsorb SOD or SOD substrates or interfere with colour detection.
		Nrf2 reporter gene assays	No known MN effects
Induction of Heat Shock Proteins		HSP Microarray or RT-PCR	No known MN effects.
		HSP western Blot	No known MN effects.
		HSP reporter gene assays	MN may interfere with fluorescence/colour detection.

Stress kinase activation		Stress kinase translocation (immunolabeling or fluorescence tagged protein expression)	MN may interfere with fluorescence/colour detection.
NO production		iNOS RT-PCR	No known MN effects.
		iNOS Western Blot	No known MN effects.
		iNOS reporter gene assays	MN may interfere with fluorescence/colour detection.
		RNS/NO Assay (DAF-DA)	MN may adsorb DAF-DA or interfere with fluorescence detection.
Inflammatory response			
Induction of pro-inflammatory peptides and cytokines, mediators, prostaglandins and leukotrienes, adhesion molecules, factors for extravasation	Blood (PBMC), co-culture models (fibroblasts, epithelial cells, macrophages/leukocytes e.g. HaCaT, CaCo-2, CaLu3, RLE-6NT, A549, NRK-52E, MDCK, HepG2, Balb/3T3, NIH 3T3, JURKAT; MonoMac, U937, THP-1, RAW-294.7) Human monocyte derived macrophages (huMDMac), Human monocyte derived dendritic cells (huMDDC) Human endothelial cells, venous, arterial,macro and microvascular, HUVEC, HASVEC, HAFEC, pulmonary microvascular EC Polymorphonuclear Granulocytes (PMN) including co-culture and flow conditions	Interleukines, chemokines and TNFα, expression by RT-PCR or microarrays, Adhesion molecule expression: E-selectin, ICAM-1, VCAM-1, etc., EIA determination	No known MN effects.
		Interleukines, chemokines and TNFα, prostaglandins and leukotrienes, secretion by ELISA	MN may adsorb Interleukines, chemokines and TNFα or interfere with (colour) detection system.
Fibrogenic effects		TGFβ, Collagen gene expression by RT-PCR	No known MN effects.
		TGFβ gene expression by ELISA	MN may adsorb TGFβ or interfere with colour detection.
Immunotoxicity		Dendritic cell maturation markers (flow cytometry).	MN may adsorb marker antibodies or interfere with fluorescence detection.
		T-cell activation (by antigen presentation), MTT Assay	MN may adsorb MTT or interfere with colour detection.
		Cell death and apoptosis by Annexin V binding/PI uptake, FACS detection.	MN may adsorb Annexin V, PI or interfere with fluorescence detection.
Blood components: Adverse effects			
Non-cellular effects	Whole blood, serum, plasma, isolated cell fractions	Fibrinogen-fibrin conversion (Thrombin), Fibrinogen	MN may adsorb coagulation proteins.

		concentration by coagulation assay	
		Fibrinolysis (Plasmin activity), D-dimer ELISA	MN may adsorb plasmin and D-dimers or interfere with colour detection.
		Complement activation (e. g. *iC3b ELISA)*	MN may adsorb iC3b or interfere with colour detection.
White blood cells		Oxidative burst (DCF-DA ROS assay)	MN may adsorb DCF-DA or interfere with fluorescence detection.
		Interleukines and cytokine expression (ELISA)	MN may adsorb Interleukines and cytokines or interfere with colour detection.
Red blood cells		Oxygen transport capacity	No standardized in vitro test available.
		Haemolysis (free haemoglobin detection)	No known MN effects.
Platelets		Contact activation (aggregometry, decrease in optical density, detected by spectroscopy)	MN may interfere with light detection.
		Thrombin generation from Prothrombin (ELISA, Thrombin activity assays)	MN may adsorb Prothrombin and Thrombin or interfere with colour detection.
		Platelet activation (Thrombin, aggregometry, decrease in optical density, detected by spectroscopy)	MN may adsorb Thrombin and interfere with light detection.
Specific endpoints			
Genotoxicity (DNA damage)	Adherent cells: HaCaT, CaCo-2, CaLu3, RLE-6NT, A549, NRK-52E, MDCK, HepG2, BEAS 2B, Balb/3T3, NIH 3T3 Suspension cells: JURKAT, MonoMac, U937, THP-1, RAW-294.7	Comet assay	No known MN effects.
		Micro Nucleus assay (FACS)	MN may interfere with fluorescence detection.
Carcinogenicity (altered gene	Immortalised mouse fibroblast	Cell Transformation Assay (CTA)	No known MN effects.

expression, malignant transformation, induction of tumor associated mutations)	(Balb/3T3, NIH 3T3); Syrian Hamster Embryo Cells (SHE)	PCR and DNA arrays	No known MN effects.
		Protein arrays	No known MN effects.
Neurotoxicity	PC12 (poor neuronal model), neuronal stem cells (not standardized), P19, SHSY-5Y	Differentiation assay (neuronal markers)	No known MN effects.
		Electrical excitability	No known MN effects.
		Neurotransmitters release (HPLC, ELISA)	MN may adsorb neurotransmitters or interfere with colour detection.
		Locomotor behaviour in embryo of zebra fish	No known MN effects.
Embryotoxicity/ Teratogenicity, developmental toxicity	Frog embryos	Frog Embryo Teratogenesis assay in Xenopus (FETAX)	No known MN effects.
	Murine stem cell line D3	Embryonal stem cell test (EST)	No known MN effects.
Phototoxicity (many nanoparticles absorb or scatter light and influence these tests)	Skin derived cells (HaCaT), fibroblasts (Balb/3T3, NIH 3T3)	DNA damage by CFE + UV exposure (anti-CPDs and anti64 PPs immunostaining)	MN may adsorb marker antibodies or interfere with fluorescence detection.
		TG 432 human skin phototoxicity test (Neutral Red uptake)	MN may adsorb Neutral Red or interfere with colour detection.
Organ specific toxicity	Primary hepatocytes	Albumin expression, p450 function, cell viability.	MN may interfere with cell viability assays.
***In vitro* barrier tests**			
Effects on biological barriers (Lung, gastrointestinal, dermal, endothelial) Tight epithelial layers Tight endothelial layers Cell membranes Blood brain barrier (BBB) Placenta Neuronal transport Blood testis	Epithelial and endothelial cell lines forming tight layers: MDCK, CaCo2, NRK, Calu-3, A549, HaCaT Endothelial cells: primary rat brain endothelial cells, hCMEC/D3, primary human lung endothelial cells Blood-Brain Barrier: primary rat or porc brain endothelial cells or hCMEC/D3 in coculture with	Trans Epithelial Electrical Resistance (TEER) = Impedance sensing	No known MN effects.
		Optical methods e.g. Digital Holography for cell morphology alterations	No known MN effects.
		Labelled MN uptake, absorption and transmigration.	Depends on the availability and detection systems of/for labelled

	astrocytes and/or pericytes Placenta: BeWo cell line or HUVEC cell line For neuronal transport and blood test is assessment are currently no suitable in vitro models available.		MN.
		Endothelial and epithelial barrier function (permeability): Evans blue albumin (EBA) and sodium fluorescein (SF) transfer in the presence of nanoparticles. Permeability and leukocyte diapedesis monitoring by EC cultures in Transwell chambers	MN may adsorb dyes or interfere with fluorescence detection.
		Endothelial and epithelial proliferation and viability, detection of cellular ATP, PI test and/or MTS assay and/or Alamar blue test	MN may adsorb dyes or interfere with fluorescence/colour detection.
		Endothelial apoptosis, Caspase-3 cleavage	No known MN effects.
		Expression of cell adhesion molecules (ICAM-1 and VCAM-1) on human brain endothelial cells, Flow cytometry assay of VCAM-1 and ICAM-1	MN may adsorb marker antibodies or interfere with fluorescence detection.
		Modulation of transport processes, Calcein assay, Digoxin bidirectional transport interaction, Hoechst assay, membrane based transporter assays	MN may adsorb dyes or interfere with fluorescence detection.

Setting Priorities for Drinking Water Contaminants (1999)
Commission on Geosciences, Environment and Resources (CGER)

http://www.nap.edu/openbook.php?record_id=6294&page=21

Summary and Application to Safer Consumer Product Alternatives Regulation, Chemical Prioritization Process

Review of Chemical Prioritization Schemes

The committee selected a total of 10 schemes (several at the recommendation of EPA) for evaluation; these are listed in Table 2-1.

- The first three (the Cadmus approach, the American Water Works Association screening process, and the Regulation Development Process) are prioritization schemes specifically intended for drinking water contaminants.
- The next three (Waste Minimization Prioritization Tool, section 4[e] of the Toxics Substances Control Act [TSCA], and California EPA Proposition 65), are general prioritization tools for environmental contaminants.
- The remaining four (the Hazard Ranking System; Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) priority listing; Hazard Analysis of Releases Inventory, and the Pesticide Leaching Potential Index), are prioritization tools for specific environmental sites or media.

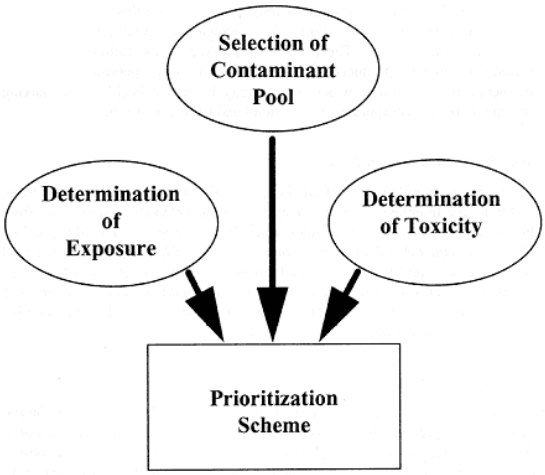
The First 6 schemes are most relevant to our evaluation of the SCPA Regulation Chemical Prioritization Scheme. They are outlined in the attached Excel document.

TABLE 2-1 Representative Chemical Prioritization Schemes and Sources

Contaminant Prioritization Schemes Reviewed	Agency, industry, or act responsible for the development of the ranking scheme.	**Contaminant Prioritization Function**
Cadmus Risk Index Approach	Cadmus Group (Cadmus Group, 1992)	Drinking water contaminants
American Water Works Association Screening Process	AWWA (RCG et al., 1993)	Drinking water contaminants
Proposed Regulation Development Process	AWWA, National Association of Water Companies, Association of Metropolitan Water Agencies, and Association of State Drinking Water Administrators (Cook, 1998)	Drinking water contaminants
Waste Minimization Prioritization Tool	EPA Office of Solid Waste and Emergency Response and Office of Pollution Prevention and Toxics (EPA, 1997)	All potential environmental contaminants
Section 4(e) of Toxics Substances Control Act	Interagency Testing Committee (Walker and Brink, 1989; Walker, 1991; Walker, 1995)	All potential environmental contaminants
State of California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65)	California Environmental Protection Agency (OEHHA, 1997)	All potential environmental contaminants
Hazard Ranking System	EPA (CFR, 1997)	Hazardous waste sites

Comprehensive Environmental Response, Compensation, and Liability Act Priority List of Hazardous Substances	Agency for Toxic Substances and Disease Registry and EPA (ATSDR, 1996)	Hazardous materials
Sediment Contaminant Inventory Hazard Analysis of Releases Inventory	EPA Office of Science and Technology (EPA, 1996)	Sediment contaminants
Pesticide Leaching Potential	EPA Office of Pesticide Programs (Wolf, 1996)	Pesticides

Framework for evaluating existing chemical ranking schemes.



- "Selection of contaminant pool" refers to the universe of chemicals considered in each prioritization scheme.
    - *What was the "bigger list" (aka "Chemicals Under Consideration" list) that we should draw from?*
- "Determination of exposure" refers to the factors considered in evaluating the extent to which ecosystem and human receptors become exposed to the contaminant.
    - *Volume in commerce, potential for exposure (defined in SB 509)*
- "Determination of toxicity" refers to the factors considered in evaluating the potential human health effects of the contaminant.
    - *AB 1879: traits, characteristics and endpoints ; potential effects on sensitive subpopulations*
- "Prioritization scheme" refers to the means by which exposure and toxicity are combined to provide a metric for ranking or prioritizing the contaminant.
    - *AB 1879 ID and prioritization process: 1) volume of chemical in commerce, 2) potential for exposure, 3) potential effects on sensitive subpopulations.*
        - *Integration with product prioritization: 1) n/a, 2) interface with product, 3) product use by sensitive populations*
    - *Should chemical/product filters be applied concurrently, sequentially? Should exposure/toxicity be applied concurrently, sequentially?*

First 7 Schemes are outlined in excel document

Summary:

Exposure:

- The exposure potential for a contaminant is determined by the likelihood of its release to the environment, the quantity released, the persistence in the environment, the proximity of the source to receptors, and the mechanisms governing its transport through the environment to a receptor. The characterization of exposure is accomplished either in an observational or a predictive fashion.
    - *Our regulations also need to take into account exposure as it relates to products, and specifically exposure to sensitive subpopulations.*

Toxicity:

- For drinking water contaminants, prioritization according to human health impacts is relevant, whereas ecosystem impacts are not. For the prioritization schemes that account for human health impacts, toxicity is quantified using measures that indicate both cancer and noncancer effects. Typically, these are cancer slope factors and reference doses, both associated with ingestion exposure, that are taken from sources such as EPA's IRIS database. More qualitative indicators include the EPA weight-of-evidence classification scheme for carcinogenicity.

Prioritization:

- Additive (either/or): Some schemes, such as the CERCLA hazardous substance prioritization scheme, use an additive approach in which some measure of a contaminant's exposure potential is added to a measure of the contaminant's toxicity. This is equivalent to an "either/or" conceptualization in which a contaminant may rank high if it has either high exposure potential or high toxicity.
- Multipicative (and): The majority of schemes use a multiplicative approach, in which a measure of a contaminant's exposure potential is multiplied by a measure of the contaminant's toxicity. This is equivalent to an "and" conceptualization in which a contaminant can rank high only if it has an appreciable exposure potential and an appreciable toxicity.

Whether an additive or a multiplicative approach is appropriate is entirely dependent on the objectives of the prioritization scheme. The difference between the approaches is most apparent when considering contaminants that rank high only in one category (either high exposure potential or high hazard)

- *AB 1879 requires that we do BOTH. It does not say that either hazard or exposure should be weighted more heavily.*

Insufficient data issue:

- Quantitative risk-ranking schemes rely on complete, high quality data for both exposure potential and toxicity. (WMPT, the HRS, the CERCLA priority listing, the Cadmus approach, HAZREL, the Pesticide Leaching Potential, and Section 4(e) of TSCA.)

  - Pro: These schemes are extremely useful for processing large quantities of data for well-characterized contaminants, and they do not rely extensively on subjective expert judgment, which may be biased or inadequate.
  - Con: However, in most cases, if a contaminant is missing critical data it either is not ranked or it drops to the lowest priority.
- Subjective: More qualitative process, with focus on data quality. (The AWWA screening process, the proposed Regulation Development Process, and California Proposition 65)
  - Do not necessarily rank contaminants, but may simply categorize them in groups with high and low priority for further action.
  - Heavily on expert judgment to make decisions in the face of uncertainty about exposure potential and health effects

Proposed Decision making framework:

For each contaminant on the CCL, there are three possible outcomes of EPA's decision process:

1. *Consider for immediate regulatory action*, as required by the Safe Drinking Water Act (SDWA) Amendments of 1996, if information is sufficient to judge that a contaminant ''may adversely affect public health" and is known or is substantially likely to occur in public water systems with a frequency and at levels that pose a threat to public health.
2. *Drop from the CCL* if information is sufficient to determine that the contaminant does not pose a risk to public health in drinking water.
3. *Conduct additional research* on health effects and/or exposure if information is insufficient to determine whether the contaminant should be regulated.

The steps in the decision process are as follows:

1. Gather and analyze available health effects, exposure, and treatment and analytical methods data for each contaminant (SB509)
2. Conduct a preliminary risk assessment for each contaminant based on the available data.
3. Issue a decision document for each contaminant describing the outcome of the preliminary risk assessment (i.e., whether the contaminant will be considered for regulation, dropped from the CCL, or retained on the CCL pending further research).
4. Issue a health advisory for each contaminant not dropped from the CCL after the preliminary risk assessment.
5. Begin compiling a regulatory package or conducting research for each contaminant remaining on the CCL after the preliminary risk assessment.