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Grant agreement number 2009 21 01

NANOGENOTOX

European Project

« Safety evaluation of manufactured nanomaterials by characterisation of potential genotoxic hazard »

Paris – January 2011

Executive Agency for Health and Consumers

Context of the initiative

- Call for Joint Action on “the Safety of Nanomaterials” – February 26th
- Work Plan for 2009 of the second programme of Community action in the field of health (2008 to 2013)
- « *Nanosafety for Success* » - DG SANCO 2nd workshop (october 2008)
  initial proposal by France (uncertainties, lack of data)

Joint Action – Priorities and Synergy

- **Synergy** with other activities
  - **OCDE** sponsorship program
  - **ISO TC229**
  - Strong interaction with **all participants**

Joint Action – Actors

- **Main Partner:** Afsset ➔ ANSES now **(Fr)**

French Agency for Food, Environmental and Occupational Health & Safety

- **16 Associated Partners (11 countries):**

  ISS(**IT**), CLMC/IMB-BAS (**BULG**), FIOH (**Fin**), NRCWE(**DK**), BfR (**DE**), NIOM (**PL**), RIVM (**NL**), UAB (**ESP**), VAR/IPH(**BE**), INSA (**PT**), and ANSES / IPL / INRS / CEA (**FR**)

- **12 collaborating partners:**

  7 ministries (**FR, IT, NL, DE, FI, ESP, BE**)

  5 Institutes JRC (**CE**), HPA (**UK**), UCD (**IR**), LNE (**FR)**, AFSSAPS **(FR)**

Joint Action – Objectives

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- To build a robust methodology (sensitive and specific) with alternative test for determining genotoxic hazard of NMs by using a ring test(s)

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Joint Action – Objectives

- To build a robust methodology (sensitive and specific) with alternative test for determining genotoxic hazard of NMs by using a ring test(s)

- Genotoxicity testing on 14 MNs commercially available
  - CNT (6)
  - TiO_2 (4)
  - SiO_2 (4)

SiO_2 available

NM-series number	Sample Ref.	process	Use	Spe. Surf. m2/g	pH	Primary particle
NM-200	PR-A-02	**Pre.**	**Food**	**220**	**7**	**5-35**
NM-201	PR-B-01	**Pre.**	**Rubber**	**160**	**6,5**	**10-15**
NM-202	PY-AB-03	**Pyr.**	**Both**	**200**	**4,2**	**submitted later**
NM-203	PY-A-04	**Pyr.**	**Food**	**200 +/- 25**	**4,2 +/- 0,5**	**approx. 12**

TiO_2 available

NM-series number	Size nm	Cryst.	Use
NM-102	**15-25**, spherical	Anatase	Photocatalytic effects, Denox
NM-103	**20**, spherical	Rutile	Cosmetics
NM-104	**20**, spherical	Rutile	Cosmetics
NM-105	**22**, spherical,	85% anatase, 15% rutile	Photocatalytic effects

CNTs available

NM-series number	Type	Use
NM-400	MWCNT	structural composites and energy applications
NM-401	MWCNT	longer
NM-402	MWCNT	structural composites and energy applications
	MWCNT	structural composites and energy applications
	MWCNT	energy/ Lithium/ion battery
	SWCNT	electronics and composites
	MWCNT	

Joint Action – Objectives

- 4 experimental steps:
  - Characterisation (PC) + protocol for dispersion
  - Toxicokinetics
  - Genotoxicity *in vitro*
  - Genotoxicity *in vivo*

Joint Action – Testing

- **Characterisation:** NRCWE (DK)
  - SOP for full characterisation of NMs including MN suspension in test media
  - SOP according state of the art (OCDE/ISO)

Various and complementary techniques used for characterisation

(TEM, X-rays diffraction, BET analysis & SAXS, RAMAN spectroscopy , thermogravimetry, Zeta potential, dustiness,...)

Dispersion protocol
Distilled water + BSA then sonication

Final protocol for producing suitable manufactured nanomaterial exposure media (SOP, Oct 2011)
http://www.nanogenotox.eu/files/PDF/web%20nanogenotox%20dispersion%20protocol.pdf

Joint Action – Testing

- ***In vitro* genotoxicity:** FiOH (FI)
  - Tests will follow the available international guidance documents
  - Comet + micronucleus assays

    ➔ Route exposure with different cell lines: pulmonary, intestinal for all MNs and human skin model for TiO_2
  - Standard tests (MLA and micronucleus assay on Human lymphocytes
  - A ring test with the most promising assay(s) on selected MN (depending on results obtained for assays and characterisation package)

Cell models

Lung: 16-HBE, BEAS 2B and A 549
Intestine: Caco2
Skin: Normal Human keratinocytes (3D-models of RHE)

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- **Toxicokinetics:** RIVM (NL)
  - Performed **before** *in vivo* genotoxicity testing
  - Oral route (Gavage) and IV for TiO2 and SiO2, only IV for CNT
  - Acute (5 days) and repeated doses (90 days)
  - Dose range finding for genotoxicity tests
  - Development of sample preparation and detection method.
  - Determination of target organ for MN accumulation and genotoxicity tests

Joint Action – Testing

- ***In vivo* genotoxicity:** ANSES (Fr)
  - On rat, 3 doses, 5 animals/dose,
  - Route of exposure: oral and instillation
  - Comet assay: maximum of 5 organs investigated
  - Comet assay performed with & w/o FpG

Goal: Correlation with results obtained *in vitro* tests and toxicokinetics

Joint Action – To Learn More

- In addition of the 4 scientific work packages: coordination, dissemination and evaluation
- Joint action approved in July 2009 – 6.2 millions of Euros and 46% funded by EC.
- Start in March 2010, for 3 years

Coordinator:

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Joint Action – To Learn More

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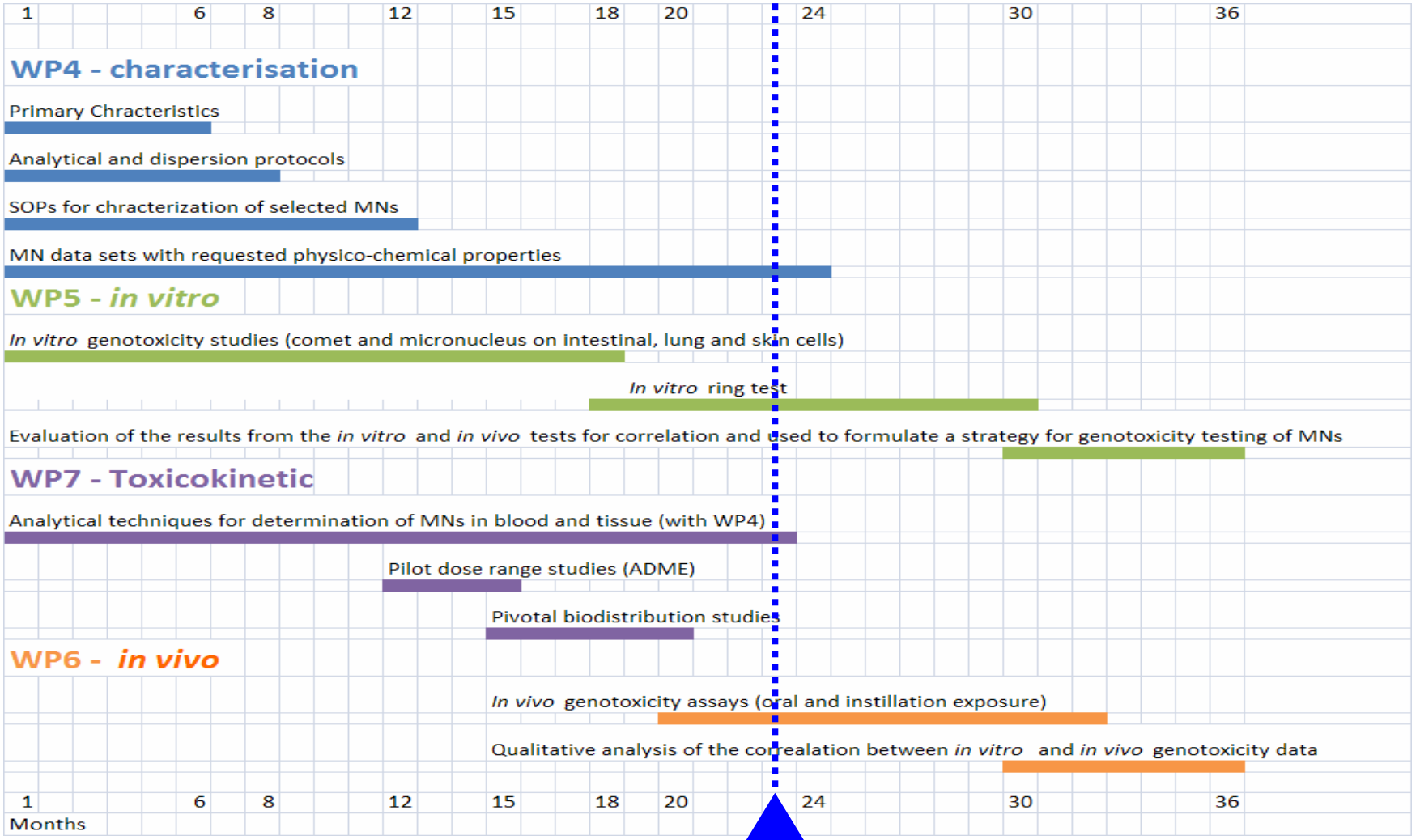

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Joint Action – To Learn More

- Up to date,
  - Dispersion Protocol fixed and validated
  - TK studies in progress,
  - Harmonisation of experimental conditions,
  - *in vitro* genotoxicity tests finished: data should now be compiled
  - *in vivo* genotoxicity tests: just started