1. Programme title

Environmental risks

2. Programme director

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3. Partners

Cluster	Role	Name (affiliation)
Cluster I Methods & tools for analysis of MNP in environmental matrices	Clusterleader	Prof.dr. P. de Voogt (UvA/KWR)
	Industries	Dr. P. Krystek (Philips/MiPlaza)
		Dr. A. van Wezel(KWR)
	Academic groups	Prof.dr. P. Schoenmakers (UvA)
		Prof.dr. W. van Riemsdijk (WUR)
		Dr. P. Leonards (VU/IVM)
Cluster II Understanding environmentalfate processes of MNP	Clusterleader	Prof.dr. W. van Riemsdijk (WUR)
	Industries	Dr. A. van Wezel (KWR)
	Academic groups & Institutes	Dr. N. van den Brink (Alterra/WUR)
		Prof.dr. K. Kalbitz (UvA)
Cluster III Modelling emission routes and environmental concentrations of MNP	Clusterleader	Prof.dr. B. Koelmans (WUR/Imares)
	Industries	Dr. J. Hofman (KWR)
	Academic groups & Institutes	Dr. P. Tromp (TNO)
		Dr. E. Roex (Deltares)
		Prof.dr. R. Laane (Deltares/UvA)
		Prof.dr. D. van de Meent (RIVM/RUN)
Cluster IV Accumulation of MNP in organisms, (eco)toxicity and mode of toxicological action	Clusterleader	Dr. J. Legler (IVM/VU) & Prof.dr. J. Hendriks (RUN)
	Industries	Dr. R. van der Oost (Waternet)
		Dr. T. ter Laak (KWR)
	Academic groups & Institutes	Dr. N van den Brink (Alterra/WUR)
		Dr. E. Roex (Deltares)
		Dr. J. Hermens (IRAS/UU)
		Prof.dr. S. Speller (RUN)
		Prof. dr. D. van de Meent (RIVM/RUN)
Cluster V Integration: adapting environmental risk assessment for MNP	Clusterleader	Prof. dr. D. van de Meent (RIVM/RUN)
	Industries	Drs. N. Zantkuijl (VEWIN)
	Academic groups & Institutes	Prof.dr. C. ter Braak (WUR/Biometris)
		Dr. H. Marvin (RIKILT/WUR)

Deltares
Institute for Environmental studies
Imares
Joint Dutch Waterworks
KWR Watercycle Research Institute
Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek (TNO)
Philips, Miplaza
Radboud Universiteit Nijmegen, Environmental Science
Radboud Universiteit Nijmegen, Institute for Molecules and Materials
Rijksinstituut voor volksgezondheid en milieu (RIVM)
University of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics
Utrecht University, Institute of Risk Assessment Sciences
VEWIN
Wageningen University and Research Centre, Biometris
Wageningen University and Research Centre, Alterra
Wageningen University and Research Centre, Aquatic Ecology and Water Quality Management
Wageningen University and Research Centre, Soil chemistry and chemical soil quality
Wageningen University and Research Centre, Institute for food safety (RIKILT)
Waternet

4. Summary

This programme aims at understanding and assessing potential environmental risks posed by manufactured nanoparticles and nanomaterials.
Nanotechnology has enormous technological and economic potential. To take full economic and societal advantage of these opportunities, it is crucial to understand and effectively manage potential environmental risks posed by manufactured nanoparticles (MNP). Any misconception on environmental risks may seriously hamper application of nanotechnology.
Currently, understanding on environmental risks posed by MNP is largely lacking. Due to the specific properties of MNP, it is expected that existing models and concepts to describe and predict environmental risks for 'normal' chemicals do not apply for MNP. This programme aims at understanding and predicting emission routes, environmental fate processes, exposure of organisms in the ecosystem, and (eco)toxicity of nanoparticles. Analytical methods to determine MNP in relevant environmental matrices are currently lacking, development and application of these methods essential to gain the desired knowledge. As integration of this programme, current paradigms for ecological risk assessment will be adapted to MNP.

5. General description of the programme

Nanotechnology has enormous technological and economic potential. To take full economic and societal advantage of these opportunities, it is crucial to understand and effectively manage potential environmental risks posed by manufactured nanoparticles (MNP). Any misconception on environmental risks may seriously hamper application of nanotechnology.

This programme aims at understanding and assessing potential **environmental** risks posed by manufactured nanoparticles and nanomaterials.
Insight into and understanding of the potential environmental risks of manufactured nanoparticles (MNP) is a crucial constraint for the societal acceptance of its many potential innovative applications and essential for safe use of innovative products based on MNP.
Several recent reviews show that currently understanding of factors governing environmental risks of nanoparticles is largely lacking, despite the fact that applications of MNP are growing fast. Knowledge gaps are:

- analytical methods to measure MNP in environmental matrices

- information of emission routes are of MNP to the environment
- understanding of the environmental fate of MNP and the role of colloidal interactions to describe their fate
- mechanisms of accumulation of MNP in organisms and mode of toxicological action and ecotoxicity of MNP.

These fundamental knowledge gaps have to be filled by a co-ordinated programme in order to make subsequent compound-by-compound testing of MNP by industry more efficient.

Manufactured nanoparticles are a large group of carbon-based or inorganic compounds with varying properties dependent on their functionalization. This programme aims at understanding and predicting *environmental exposure* and *adverse effects* of various classes of nanomaterials, and adapting existing risk assessment methodologies. Analytical methods to determine MNP in relevant environmental matrices are currently lacking, development and application of these methods essential to gain the desired knowledge. As integration of this programme, current paradigms for ecological risk assessment will be adapted to MNP.
The central questions in this programme are examined in five clusters

1. Methods and tools for analysis of MNP in environmental matrices
2. Understanding environmental fate processes of MNP
3. Modelling emission routes and environmental concentrations of MNP
4. Accumulation of MNP in organisms, (eco)toxicity and mode of toxicological action of MNP
5. Integration: adapting environmental risk assessment for MNP

By addressing these topics, the programme will develop new approaches and tools that provide information on groups of nanoparticles, enabling a rapid and valid differentiation between MNPs with low or high environmental risks in the early phase of development. Available risk information will be used for extrapolation between different types and functionalizations of MNP, enabling smart design of new nanoparticles.

The need for a better understanding of the risks of nanotechnology recently was stressed by the Dutch parliament. Several national institutes and academic groups currently start to work in this emerging field or environmental risk research, until now without a co-ordinated interaction. This programme is an investment in this quickly growing field and builds upon the strong Dutch scientific tradition in the field of environmental risk assessment. The programme will give special attention to links with ongoing nano-related programmes, such as within the EU framework programme, the OECD Working Party on Manufactured Nanomaterials (WPMN), the programme set by the US Environmental Protection Agency and industrial programmes (e.g. CEFIC-LRI).
The work as performed in the programme on human health risks of MNP will bring useful information for the current programme and vice-versa, examples are the work on the database with gap and cluster analysis, the work on bioavailability and mode of action of MNP. Obviously, the programme will interact with other programmes in HTS&M. The programme will specifically focus on MNP as synthesized in the nanomaterials theme and on MNP that are in focus for application in food, energy, water and medicine.
As integration of this programme, current paradigms for ecological risk assessment will be adapted to MNP. Within the context of REACH, the EU legislation on authorization of chemicals, a specific approach for MNP is under discussion. The knowledge generated in this programme will be very helpful for this European policy development.
An improved understanding of the factors that govern environmental risks of MNP will enable a more targeted testing and data generation for MNP by responsible industries possible, and thus reduce costs for testing.

Parties involved have a strong scientific background in the field of analytical chemistry, environmental (geo)chemistry, ecotoxicology, environmental risk assessment and bridging environmental science to political decision makers. PM CVs are attached.

6. Research clusters and projects

Cluster I: Methods & tools for analysis of MNP in environmental matrices
Quantification and characterization of nanoparticles and their (degradation) products is essential to determine their distribution within the environment, to relate their toxicological effects to concentrations, and to assess environmental risks. Currently, analytical methods for MNP are generally lacking for relevant matrices at relevant sensitivities. Relevant environmental matrices are water, effluents, sediment, soil, sludge, and tissues.
This cluster develops and applies innovative quantitative analytical methods to answer this need.
Depending on matrix and type of MNP studied several techniques are promising, such as asymetric flow field-flow fractionation with high resolution ICP-MS, pyrolyse GC-MS, accurate mass screening using LTQ Orbitrap MS, and tissue Maldi-Tof imaging. Static and dynamic light scattering will be used for the characterization of particle properties in dispersions. The MNP studied will partly be nanoparticles as synthesized in the nanomaterials theme and as in focus for application in the themes on food, energy, water and medicine. The cluster will also focus on first and second generation MNP that are currently available on the market.
Asymmetric flow field-flow fractionation can separate different inorganic nanosized particles in a complex environmental matrix, coupling to high resolution ICP-Mass Spectrometry (HR-ICPMS) allows analysis of very low concentrations and to measure isotopic signals. Knowledge about the surface chemical behavior of the various inorganic MNP (see cluster 2) can help to design improved conditions for separation of the MNPs of interest.
By accurate mass screening up to very high molecular masses nano-aggregates or agglomerates will be analyzed. Imaging tools (whole organism and tissue Maldi ToF imaging) provide the link between whole organism/tissue MNP distribution (lab and field) and observed effects of MNP.
A baseline field survey using the different techniques to be developed provides data on the occurrence of MNPs in the aquatic and terrestrial environment, and waste water systems.
Budget: 1400 k€
Partners: Partners: MiPlaza/Philips (300 k€), WUR Soil chemistry and chemical soil quality (50 k€); Dutch Waterworks (200 k€), UvA (100 k€), IVM/VU (50 k€); requested subsidy 700 k€

Cluster II: Understanding environmental fate processes of MNP
Insight in environmental fate of MNP needs basic information on physico-chemical properties and their behavior in various environmental matrices and interaction with naturally occurring components such as humic acids. Due to the reactive properties of MNP these interactions are expected to significantly influence their environmental behavior. The colloidal stability will strongly affect the distribution of the MNP over various environmental compartments, and thus their mobility in the environment. Innovative fate models for MNP will be a combination of insights from environmental chemistry and physical and colloidal chemistry.
We will develop, test and apply quantitative models for the interaction between naturally present ions like protons (pH), humic and fulvic acids, phosphate, calcium and heavy metals and inorganic MNP. The surface energy of manufactured metaloxide nanoparticles will be assessed as a function of environmental conditions, relevant for the stability of the particles and probably also their toxicity.
A better understanding of the fate of carbon-based MNP can be partly based on knowledge and techniques as applied for the natural carbon cycle and natural soil organic matter. The role of MNP may be similar to that of black carbon. Recalcitrance towards microbial and/or abiotic mineralization of carbon-based MNP will be studied, and their relevance for stabilization of soil organic matter and carbon turnover dynamics.

The physicochemical properties of organic MNP will depend on their functionalization. Series of MNPs with different functional groups, partly synthesized in co-operation with the theme on nano-materials, will be tested in various aqueous matrices varying in pH, ionic strength, organic dissolved matrices. Quantitative models for the interaction of organic MNP with naturally present ions and organic matrices will be developed.
At current hardly information is available on bioavailability and biomagnification hampering a proper risk assessment. This cluster relates uptake kinetics to MNP characteristics and soil properties, and assesses MNP speciation and bioavailability. We will assess a simple experimental soil food chain, and relate biomagnification to MNP physico-chemical properties.
Budget 1800 k€
Partners: WUR/Alterra (225 k€); WUR omgevingswetenschappen (325 k€); UvA (125 k€); Dutch Waterworks (225 k€); requested subsidy 900 k€

Cluster III: Modelling emission routes and environmental concentrations of MNP
Currently, information on emission routes is lacking as are models to predict the environmental concentrations in various environmental compartments. Prediction of environmental concentrations can contribute significantly to prospective risk assessment of MNP. Emissions will be predicted for current and future MNP-application combinations, and therefore be or relevance to the themes that study application of MNP in food, energy, water and medicine. Fate models adapted to MNP (developed in cluster II) will be implemented in existing water quality models to predict environmental concentrations. Furthermore, environmental emissions are studied in more depth for MNP in water treatment applications and in traffic applications.
The cluster performs mass-balance model scenario studies covering MNP emission, transport and distribution between the major environmental compartments. The modelling is supported by model-driven experimental work as described in cluster 2 and 4.
Existing water quality models will be adapted to link emissions, transport and fate to water quality. Emissions for current and future applications of MNPs to different environmental compartments will be parameterized, using a scenario-based approach and information of releases during the lifecycle and thus including uncertainty. In this way it is possible to model the impact of emission reduction measures.
Many MNP are used as consumer products and will thus enter the environment via STP. The project studies if current waste water and drinking water treatment technologies form barriers against further dispersal of MNP. There is a direct link with work as performed under the clean water theme.
MNP in relation to traffic are currently in use in various traffic applications (car tires, catalysts and coatings). As a result MNP are released in air, which is hypothesized (cf discussions on air quality related to particulate matter concentrations) to be a relevant exposure route for the general population and an important route of emission. Insights gained are coupled to the programme on human health (cluster 3). Measurements will be carried out during relevant wear and abrasion processes of these MNP applications on lab and field scale.
Budget: 1400 k€
Partners: WU/Imares (150 k€), Deltares (175 k€), Dutch Waterworks (125 k€), RIVM (25 k€), TNO (225 k€); requested subsidy 700 k€

Cluster IV: Accumulation of MNP in organisms, (eco)toxicity and mode of toxicological action
In this cluster the physical chemical properties of MNP will be linked to their accumulation and distribution on the levels of the cell and the organism. MNP partitioning into membranes, transfer across cell membranes to reach target sites, and distribution and kinetics in whole organisms are experimentally studied. Ultramicroscopic techniques will be used to study the fate of MNP in cells of aquatic organisms exposed to MNP, and tissue Maldi-Tof imaging information as developed in cluster I will be of relevance. The MNP studied will partly be nanoparticles as synthesized in the nanomaterials theme and as in focus for application in the themes on food, energy, water and

medicine. The cluster will also focus on first and second generation MNP that are currently available on the market. Insights gained are coupled to the programme on human health risks (cluster 4).
The MNP behavior in biologic systems will both be linked to various *in vivo* and *in vitro* toxicity studies on adverse effects on gene, cell and organism level (e.g. using gene-arrays, Q-PCR, aquatic and terrestrial invertebrates and fish toxicity studies). Dose-effect studies of MNP at different levels of biological organization give insight into the type of adverse effects and lead to understanding the mode of toxicological action.
With this information gained, existing accumulation models (e.g. SimpleBox/OMEGA) will be modified by incorporating transport and kinetic processes of MNP by organisms from literature and studies within this project. This will provide tools to extrapolate to other MNP and interprete accumulation and distribution and effects based on the properties of the MNP in question.
Budget: 1600 k€
Partners: WUR/Alterra (125 k€); Waternet (100 k€), VU/IVM (125 k€), IRAS (75 k€), Dutch Waterworks (150 k€), RUN (175 k€), RIVM (50 k€) ; requested subsidy 800 k€

Cluster V: Integration: adapting environmental risk assessment for MNP
In this cluster, current paradigms for ecological risk assessment will be adapted to MNP making scientific uncertainties explicit. The knowledge gained in clusters 2, 3 and 4 on understanding and predicting *environmental exposure* and *adverse effects* of various classes of MNP will be integrated by adapting existing risk assessment methodologies for MNP.
The ecological risk assessment of MNP differs from current risk assessment of chemical substances, as current risk assessment is based on the paradigm that the active species is the molecular form. For MNP however, the processes that control their environmental concentrations have not even adequately been identified. Colloid science predicts that MNP behavior is a result of aggregation/agglomeration and settling/resuspension, rather than from thermodynamic equilibrium partitioning. This cluster will critically and systematically review existing modeling procedures and propose alternate assessment procedures and models to make (EU) risk assessment fit to cope with MNP. The methodology will be adapted to the special needs, requirements and uncertainties imposed by MNP, using probabilistic techniques for risk assessment.
The large pool of data on physico-chemical properties, environmental behavior and ecotoxicological effects of MNP, generated in this programme, other themes and in literature needs to be collected and organized for meta-analysis. Good design and development of a knowledge base will help to design intelligent testing strategies, provide advice to regulators, to identify knowledge gaps and enabling manufacturers in smart design of new nanoparticles. There is a direct link of this activity to cluster 1 in the programme on human health risks.
Budget: 1300 k€
Partners: RIVM (150 k€), RUN 145 (k€), VEWIN (30 k€), WUR/Biometris (200 k€), RIKILT (125 k€); requested subsidy 650 k€

7. Knowledge transfer

Knowledge generated in the different projects will be disseminated through publication of scientific papers, conferences and symposia.
Knowledge transfer within this programme will be organized by periodical steering meetings with all clusterleaders, and periodical scientific meeting with all researchers involved.
To promote the implementation and use of developed approaches and tools by the various stakeholders, existing contacts with e.g. VNO-NCW, VNCI, OECD, and relevant ministries and directorates will be employed. To this purpose, results of the programme will be presented in such a way that it is useful for policy making by authorities and industry, eg via the VNO platform on risks of nanotechnology and via the interdepartmental group on this subject. The results will furthermore be directly coupled to the policy-makers involved with design of legislation on MNP.
Specific attention will be given to active collaboration with other HTSM-themes and programmes, as described throughout this programme.

In order to provide stakeholders with an integrated overview and analysis of scientific results generated within and outside this FES proposal, regular reporting will be linked to the yearly signalling reports of KIR-nano.
In order to stimulate students in e.g. life sciences and product development to integrate risk related research in their way of thinking on sustainable innovations, links with educational programmes are explored.

Investments
Asymetric flow field-flow fractionation

8. Overview of milestones and deliverables

Milestones & Deliverables			
Number	**Description**	**Project(s)**	**Month**
D1	Quantitative analytical method asymetric flow field-flow fractionation with high resolution ICP-MS	I	18
D2	Quantitative analytical method accurate mass screening using LTQ Orbitrap MS	I	12
D3	Quantitative analytical method tissue Maldi-Tof imaging	I	18
D4	Baseline field survey using the developed analytical techniques on occurrence of MNP in the environment	I	42
D5	Innovative fate models for MNP	II	32
D6	Test the interaction between naturally present ions like protons (pH), humic and fulvic acids, phosphate, calcium and heavy metals and inorganic MNP	II	24
D7	Test recalcitrance towards microbial and/or abiotic mineralization of carbon-based MNP, and relevance for carbon turnover dynamics	II	36
D8	Test series of MNPs with different functional groups in various aqueous matrices	II	24
D9	Assess a soil food chain, and relate biomagnification to MNP physico-chemical properties.	II	36
D10	Implement fate models in water quality models to predict environmental concentrations	III	36
D11	Scenario-based approach on emissions related to future applications of MNP	III	42
D12	Model impact of emission reduction measures.	III	42
D13	Test barrier function of current waste water and drinking water treatment technologies for dispersal of MNP	III	36
D14	Air measurements resulting from traffic related MNP applications on lab and field scale	III	30
D15	Apply ultramicroscopic techniques to study the fate of MNP in cells of aquatic organisms	IV	30
D16	Tox studies on adverse effects on using gene-arrays and Q-PCR	IV	30

D17	Test MNP partitioning into and transfer across membranes	IV	36
D18	Test distribution and kinetics in whole organisms and relate to in vivo toxicity studies	IV	36
D19	Modify existing accumulation models by incorporating transport and kinetic processes of MNP	IV	42
D20	Adapt current paradigms for ecological risk assessment to MNP	V	48
D21	Use probabilistic techniques for risk assessment to make uncertainties explicit	V	48
D22	Inventory of ongoing world wide initiatives and availability of data on ecological safety assessment of MNP	V	12
D23	Design of meta database	V	18
D24	Identify knowledge gaps using meta database	V	42
D25	Design intelligent testing strategies	V	42

All deliverables will be described in scientific papers, in total at least 40. Furthermore , aimed at policy-makers a yearly integrated report on the results will be prepared.