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Nanomaterials under REACH

Nanosilver as a case study

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Abstract

Nanomaterials under REACH
Nanosilver as a case study

Some adjustments are needed in the European chemicals legislation REACH to assess and control the risks of nanomaterials. The information on substances to be provided under REACH is not sufficient to determine the specific properties of nanomaterials, nor to assess how these properties affect their behaviour and effects in humans and the environment. RIVM concluded this following research into the suitability of REACH for nanomaterials. RIVM therefore proposes an adapted set of minimum information requirements, to be applied to all nanomaterials to be registered under REACH, independent of their volume of production and import. These requirements allow a risk assessment of nanomaterials.

Over the last years the use of nanomaterials has strongly increased. As yet, nanomaterials are defined as substances of which the discrete parts have at least one dimension smaller than one hundred nanometres. Due to their nanosize they have specific properties. Legislation should focus on controlling the potential hazards and risks of these nanomaterials.

By conducting a hypothetical registration of nanosilver it was investigated whether REACH is suitable for assessing the safe use of nanomaterials. From this it appeared that no definition of a nanomaterial is present, and that a relevant measure for expressing harmfulness and exposure is as yet not known. In addition, the standard information requirements are insufficient to assess hazard and exposure. They are also insufficient for a proper characterisation of the nanomaterial. Consequently, it cannot be determined to what extent the nanoform of a substance corresponds to the non-nanoform of the same substance. Furthermore, it is unclear whether current risk reduction measures and extrapolation methods in risk assessment, as established for non-nanomaterials, are applicable to nanomaterials.

Key words: REACH, nanomaterials, nanosilver, risk assessment

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Rapport in het kort

Nanomaterialen onder REACH
Nanozilver als een voorbeeldstudie

Om de risico's van nanomaterialen te kunnen inschatten en beheersen, zijn enkele aanpassingen nodig in de Europese chemicaliënwetgeving REACH. De gegevens over stoffen waar REACH standaard om vraagt, zijn namelijk onvoldoende om de specifieke eigenschappen van nanomaterialen te bepalen. Hetzelfde geldt voor het bepalen van de invloed van deze eigenschappen op het gedrag en de effecten van nanomaterialen in mens en milieu. Dit blijkt uit onderzoek van RIVM naar de geschiktheid van REACH voor nanomaterialen. Het instituut stelt daarom een aangepaste set minimum informatievereisten voor, voor alle te registreren nanomaterialen onder REACH, ongeacht de omvang van productie en import. Deze vereisten maken het mogelijk de risico's van nanomaterialen te beoordelen.

Het gebruik van nanomaterialen neemt de laatste jaren sterk toe. Nanomaterialen worden vooralsnog gedefinieerd als stoffen waarvan de deeltjes minstens één dimensie kleiner dan honderd nanometer hebben. Vanwege hun afmeting hebben ze specifieke eigenschappen. Wetgeving moet erop gericht zijn de potentiële gevaren en risico's van deze nanomaterialen te beheersen.

Aan de hand van een hypothetische registratie van nanozilver is onderzocht of REACH geschikt is om een veilig gebruik van nanomaterialen vast te stellen. Hieruit bleek onder andere dat een definitie van nanomateriaal ontbreekt, en dat de juiste maateenheid om de schadelijkheid en blootstelling in uit te drukken nog niet bekend is. Ook is de verplichte standaardinformatie ontoereikend om de blootstelling en gevaren in te kunnen schatten, en om het nanomateriaal goed te kunnen karakteriseren. Mede door de laatste beperking is niet vast te stellen in hoeverre de nanovorm van een stof overeenkomt met de niet-nanovorm van dezelfde stof. Bovendien is het onduidelijk of de huidige extrapolatiemethoden in de risicobeoordeling en de maatregelen om risico's te beheersen geschikt zijn voor nanomaterialen. Deze methoden en maatregelen zijn immers vastgesteld voor niet-nanomaterialen.

Trefwoorden: REACH, nanomaterialen, nanozilver, risicobeoordeling

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Preface

This report describes a hypothetical registration of nanosilver under the new EU REACH regulation on chemicals, taking into account the ongoing discussions within the REACH Competent Authorities and its Subgroup on Nanomaterials on how REACH applies to nanomaterials (as described in documents of this subgroup dated December 2008-March 2009). The case study on nanosilver is purely a scientific exercise, with the aim to generate recommendations for future policy guidance on how to deal with first generation nanomaterials under REACH. Given this, it is stressed that this report does not pretend to provide a complete overview of all available toxicity data on (nano)silver, and is as such not to be used for an actual registration under REACH.

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Summary

Due to their nano-size, nanomaterials (≤ 100 nm) have specific characteristics that may differ from non-nanomaterials. The development of nanomaterials and their increasing use in all sorts of industrial applications and consumer products, challenges the regulating authorities to develop frameworks which can adequately control the potential hazards and risks of these nanomaterials. In Europe such a framework is the new regulation of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Under REACH, manufacturers, importers and downstream users have to ensure that the substances they manufacture, place on the market or use, do not adversely affect human health or the environment. Since REACH deals with substances, in whatever size, shape or physical state, substances at the nanoscale are also covered by REACH and its provisions apply. This implies that also the safety of nanomaterials to human health and the environment should be ensured under REACH, covering their whole life cycle.

The aim of this report is two-fold. First, to investigate the suitability of REACH in ensuring the safety of nanomaterials by conducting a hypothetical registration under REACH of metallic silver, a substance that exists both in nanoform and in non-nanoform (*i.e.* bulk form). Nanosilver is a first generation nanomaterial, and the case study on nanosilver is used to examine the problems that potential registrants may encounter when trying to register such a substance under REACH. Second, to propose a risk assessment framework for first generation nanomaterials under REACH, based on the information generated in the case study.

In chapter 2 the requirements for registration of a substance under REACH are briefly described. These requirements depend on the tonnage a substance is manufactured or imported in, and may include a chemical safety assessment.

In chapter 3 an overview is presented of some recent analyses on the suitability of existing testing methods in determining the toxicity of nanomaterials. The majority of these methods appears to be suitable for nanomaterials, albeit that in many cases some modifications are needed to address nano-specific issues. These issues have been described for physicochemical, ecotoxicity and toxicity tests. The last part of chapter 3 reviews the (few) risk assessment approaches for nanomaterials that have been described so far. Due to their broad outline and lack of specifics on the kind of testing considered essential for nanomaterials, these frameworks are deemed of limited value.

In chapter 4 the results and conclusions are presented of the hypothetical registration of metallic silver (in bulk and nanoform) under REACH. The complete chemical safety assessment is presented in the Chemical Safety Report in Appendix 6. For this case study some assumptions on the tonnage, characteristics and use of nanosilver were made and only readily accessible public data were used as information source (and should as such not be seen as a complete overview of the toxicity of silver or nanosilver). The available data on silver in bulk form would in principle fulfil the information requirements under REACH for the assumed tonnage and would allow concluding on classification and labelling and a measure of dose-response. For nanosilver, however, several information gaps could be identified. First of all, a proper characterisation of nanosilver was difficult with the standard information requirements for physicochemical properties under REACH (even though some were arbitrarily chosen for the case study), which made it impossible to conclude on the sameness between the nanoform and bulk form of silver. Secondly, without information on the kinetics of dissolution and on partitioning available, it was not possible to predict the fate and behaviour of nanosilver in the environment and the human body. It is thus not clear whether its behaviour is comparable to bulk silver

or not. Consequently, the option of using data on bulk silver to fill the data gaps identified for nanosilver (*i.e.* read-across) was not considered viable in the absence of a conclusion on the sameness.

Chapter 5 presents the extrapolation of the findings from the nanosilver case study to nanomaterials in general. It is concluded that REACH is not sufficiently implementable for nanomaterials. First of all, for the scope of risk assessment of nanomaterials, a proper definition relating to nanomaterials needs to be developed. Also relevant dose metrics need to be established since mass concentration, the metric normally used for non-nanomaterials, may not be the most appropriate for nanomaterials for all endpoints. Further guidance is also needed on proper characterisation of a nanomaterial and its corresponding bulk form, and how to address different shapes, sizes and size distributions of a nanomaterial in substance identification under REACH. This is considered essential for the sameness analysis (between a nanoform and a bulk form of a substance, or between different sizes/shapes of a nanomaterial), and for the propagated use of non-testing methods like read-across under REACH. Also the use of *in vitro* (screening) methods is propagated. However, for nanomaterials there are at the moment too many knowledge gaps and *in vitro* assay protocol issues to deem this a viable option, also given the lack of proper databases to translate the *in vitro* results to the *in vivo* situation.

With respect to (eco)toxicity testing, kinetic information is currently not a standard requirement under REACH but is considered essential to both human health and environmental hazard assessment of nanomaterials. In addition, for some existing test guidelines extension of parameters to be tested may be necessary, and the influence of physicochemical characteristics of the test material on sample preparation and dosimetry should be considered as well. For a proper exposure assessment, also existing exposure models need to be adapted for use for nanomaterials, by incorporating nano-specific parameters. And finally, in order to demonstrate 'safe use', standard default assessment factors used in extrapolation procedures for non-nanomaterials need to be examined for their applicability to nanomaterials, and nano-specific operational conditions and risk management measures need to be developed to control exposure to nanomaterials.

Based on all these observations, in chapter 6 a risk assessment framework is proposed for first generation nanomaterials under REACH. In this proposal the basic requirements of the current REACH legislation still apply, with some nano-specific adaptations. It is proposed that for nanomaterials the tonnage-dependent need for registration and information requirements should be reconsidered. Instead, a base set information requirement is proposed for all nanomaterials to be registered, independent of their production/import volume. This base set covers what is considered to be the primary information needs on nanomaterials. Another recommendation is to request for all nanomaterials to be registered a technical dossier plus, independent of tonnage, a chemical safety assessment documented in a chemical safety report. This approach is proposed to be used at least for the next few years, until further investigations allow certain patterns in the behaviour of nanomaterials to be established, on the basis of which, in time, the data requirements for nanomaterials can be adapted. As discussions on the definition and dose metrics of nanomaterials are ongoing, the proposal recommends for the time being to use the following definition of a nanomaterial: 'any form of a material that is composed of discrete functional parts, many of which have one or more dimensions of the order of 100 nm or less', and to use (with caution) mass concentration as the dose metric for nanomaterials.

1 Introduction

The EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) suggests a nanomaterial to be defined as any form of a material that is composed of discrete functional parts, many of which have one or more dimensions of the order of 100 nm or less (SCENIHR, 2007a). The nano-size of nanomaterials results in specific physicochemical characteristics that may differ from those of the 'bulk' material or particles of larger size. Because of their specific properties, manufactured nanomaterials (of which there are at the moment already first, second and third generations) may bring significant innovation and advances to society and benefits for human health and the environment. At the same time, however, their specific properties may lead to different interactions in the physiology in humans and the environment and to effects that significantly differ from those known of bulk materials without such physicochemical properties. It will thus be necessary to ensure their safety for humans and the environment and to avoid negative impacts on society.

REACH is the new regulation of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals, that is operational in Europe since June 2007 (EU, 2006). Under REACH, manufacturers, importers and downstream users have to ensure that the substances they manufacture, place on the market or use do not adversely affect human health or the environment. It applies to substances on their own, in preparations or in articles. Within the context of REACH, it is of main importance for the development of nanotechnologies and nanomaterials that also their safety to human health and the environment is ensured, covering the whole life cycle. There are no provisions in REACH referring specifically to nanomaterials, nor has a definition relating to nanomaterials been laid down. But since REACH deals with substances, in whatever size, shape or physical state, substances at the nanoscale are also covered by REACH and its provisions apply (European Commission, 2008).

REACH is not the only regulatory framework dealing with the risk assessment of nanomaterials. There are several other types of legislation (like for instance the worker protection legislation) within which the risks of nanomaterials will need to be addressed as well. This report, however, will deal with REACH only, and only with the registration part. The basic principles of risk assessment, *i.e.* exposure assessment, hazard assessment and a comparison of these two aspects in risk characterisation, are the same in REACH as in any other regulatory framework. Under REACH, however, the focus of the risk assessment is on risk management, *i.e.* on the demonstration that risks are controlled for both the human target populations (workers, consumers and humans exposed via the environment) and the environmental (aquatic, terrestrial and atmospheric) compartments to be protected.

To date, most presently known nanomaterials are derivatives of existing bulk materials, which are defined as any form of a material with dimensions above 100 nm. Some nanomaterials are only produced in low volumes, whereas others are produced in high volumes, sometimes even higher than the corresponding bulk material. Interestingly, the majority of the bulk materials of nanomaterials currently on the market will have to be registered under REACH before the 1st of December 2010, because they are manufactured or imported in the EU in volumes of 1000 tonnes or more per year (or otherwise fall into other categories of substances that have to be registered by that date, *i.e.* substances classified as CMR[1] category 1 and 2 and manufactured or imported at tonnages of 1 tonne or more per year, and substances classified as very toxic to the aquatic environment and manufactured or imported

[1] CMR: carcinogenic, mutagenic or toxic to reproduction.

at tonnages of 100 tonnes or more per year). A 'sameness' analysis should indicate whether the substance at nanoscale can be considered as a specific physical form of the bulk substance (*i.e.* both are covered by the same EINECS[2] entry), or is a different substance than the bulk substance (*i.e.* separate EINECS entries, or no EINECS entry for the nanoscale substance). The outcome of this substance identification is critical, as it forms the basis for data sharing obligations and for the joint submission of a registration dossier under REACH (the one substance – one registration principle). In case a nanomaterial is identified as a nanoform of a bulk substance, data sharing obligations with the related bulk substance apply within one common SIEF[3]. In case a nanomaterial is identified as a different substance, its registration needs to be addressed in a different SIEF, with data sharing obligations within that SIEF. At the moment, however, the issue of substance identification and what kind of information plays a role in deciding on the sameness of a substance are still under discussion within the REACH Competent Authorities and its Subgroup on Nanomaterials. Also the substance definition in REACH does not provide clues for differentiating substance identities on the basis of physicochemical characteristics. Furthermore, the science of nanotechnology might not yet be advanced enough to allow in all cases an easy distinction between nanoforms and other forms of a substance. Moreover, the shape, size and subsequently the specific properties of nanomaterials may change during the cascade of production, processing, use, and emission. All this seriously complicates the 'sameness' analysis, which under REACH is to be carried out by potential registrants. Therefore, further work and guidance on the 'sameness' analysis is urgently needed, especially for nanomaterials that can be considered a specific form of a bulk material that already has to be registered before the 1st of December 2010. In that case, the registration date for the bulk material also applies to the nanomaterial. In addition, the registration dossier should include any relevant nano-specific information next to information on the bulk substance. Further work and guidance is therefore also urgently needed on (specific) information requirements for nanomaterials.

This report deals with a case study on nanosilver, a first generation nanomaterial, conducted by order of the Netherlands Ministry of Housing, Spatial Planning and the Environment (VROM). In order to examine the problems that potential registrants may encounter when trying to register a nanomaterial under REACH, a hypothetical registration of silver was conducted. Silver exists both in bulk form and in nanoform, and assumptions were made on its tonnage and on the use and size and shape of nanosilver. The objective of performing this case study was to generate information that could be helpful in formulating a risk assessment framework for first generation nanomaterials under REACH.

Following some general information on nanomaterials and REACH (chapter 2) and on (approaches for) risk assessment of nanomaterials (chapter 3), in chapter 4 the results and conclusions are presented of the hypothetical registration of nanosilver under REACH, where possible extrapolated to nanomaterials in general (chapter 5). Finally, in chapter 6 a proposal is made for an approach for risk assessment of first generation nanomaterials. This report is intended to be of help to the REACH Competent Authorities and its Subgroup on Nanomaterials in developing (guidance for) a risk assessment framework for nanomaterials under REACH, from which subsequently industry and society can benefit.

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2 EINECS: European Inventory of Existing Commercial Chemical Substances. REACH distinguishes between phase-in substances (= substance with an EINECS entry) and non-phase-in substances (= substance without an EINECS entry).

3 SIEF: Substance Information Exchange Forum.

2 REACH and its requirements for registration

Substances, including substances at the nanoscale, manufactured or imported in volumes of 1 tonne or more per year have to be registered under REACH. For the registration of a nanomaterial it is first of all important to establish whether the nanomaterial meets the criteria of a phase-in substance (REACH article 3(20)) or whether it is a non-phase-in substance[4]: only phase-in substances can benefit from extended registration deadlines, provided they have been pre-registered. Further, when a substance not only exists in the nanoform but also in the bulk form (which is true for most presently known nanomaterials), a 'sameness' analysis should make clear as to whether the substance at nanoscale can be considered as a specific physical form of the bulk substance, or is a different substance than the bulk substance. In case the nanomaterial is identified as a nanoform of a bulk substance, obligations for data sharing and joint submission of a registration dossier apply. In case a nanomaterial is identified as a different substance, data cannot be shared easily and the registration should be dealt with separately.

The requirements for registration under REACH are described in the following paragraphs (based on EU, 2006; European Commission, 2008). It is to be noted that the registration of a substance existing in the nanoform as well as in the bulk form can be complex, because not only the information of the substance in the bulk form should be included in the registration dossier, but also any information regarding intrinsic properties where the properties of a substance in the nanoform differs from the bulk form, any different classification and labelling, any different chemicals safety assessment as well as all identified uses and relevant exposure scenarios for the nanoform of the substance. With the separate registration of a nanomaterial, the production/import volume is of extreme importance, since data requirements for registration are tonnage-dependent. For nanomaterials produced/imported in quantities below 1 tonne per year, no safety testing is required under REACH. However, when produced/imported in quantities of 1000 tonnes or more per year, a full (eco)toxicological data set is required (see Appendix 1).

2.1 Gathering and generating information

All available and relevant information (Annex VI of REACH) on the substance, regardless of whether testing for a given endpoint is required or not at the specific tonnage level, should be gathered and considered for the registration. In addition, information on exposure, use and risk management measures should be provided. Based on the information required for registration (Annexes VI and VII–XI), further testing may be needed. A registrant may decide that he needs to generate further information beyond the information required through Annexes VII–X of REACH (see Appendix 1) in order to be able to demonstrate and document that the risks of the substance (in all its forms) are controlled. This also applies to nanomaterials which have specific properties that may not in all cases be covered by the endpoints currently included in the REACH annexes.

The information requirements increase with the tonnage manufactured or imported. The tonnage triggers for registration apply to the total volume of a substance manufactured or imported by a

[4] A phase-in substance is a substance which has been listed in EINECS in the past and was considered an existing substance before the entry into force of REACH. A non-phase-in substance is a substance which has no EINECS entry and was considered a new substance before the entry into force of REACH.

registrant. Thus, for substances which exist both in a conventional form and in a nanoform, and will be covered in one registration, the total volume determines the information requirements.

2.2 Substance identification

Any substance needs to be identified by a combination of the appropriate identification parameters (Annex VI of REACH): name or other identifier of the substance, information related to molecular and structural formula and composition of the substance. Under REACH, a substance is defined as 'a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive[5] necessary to preserve its stability and any impurity[6] deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition'. Based on the chemical composition, the registrant has to decide whether the substance to be registered is either 1) a mono-constituent substance[7], 2) a multi-constituent substance[8], 3) a mono- or multi-constituent substance needing some additional physical parameters for proper characterisation, or 4) a UVCB substance[9]. The substance identification information should allow a 'sameness' analysis, at least for bulk substances. Nanomaterials, for which the discussion on the definition is still ongoing, probably belong to category 3) and thus may need further parameters/descriptors for proper characterisation, such as for example those mentioned by OECD[10] in their Sponsorship Programme (see section 3.1, and Appendix 2 under 'Nanomaterial information/ identification' and 'Physical-chemical properties and material characterization'), although these partly go beyond the current REACH requirements. Additional considerations such as different physicochemical, toxicological and ecotoxicological properties may in certain cases play a role in deciding on the sameness of the substance.

2.3 Classification and labelling

A substance with different sizes or forms can have different classifications, as for example is the case for nickel powder (particle diameter < 1 mm) and nickel. So, when it comes to evaluating the available information for the purposes of classification, one shall consider the forms or physical states in which the substance or mixture is placed on the market and in which it can reasonably expected to be used.

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5 An additive is defined as a 'substance that has been intentionally added to stabilise the substance'.

6 An impurity is defined as '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'. Normally, impurities present in a concentration ≥ 1 % should be specified. However, impurities that are relevant for the classification and/or for PBT assessment shall always be specified.

7 A mono-constituent substance is a substance in which one main constituent (not being an additive or impurity) is present to at least 80 % (w/w).

8 A multi-constituent substance is a substance in which more than one main constituent (not being an additive or impurity) is present in a concentration ≥ 10 % (w/w) and < 80 % (w/w).

9 Substance of Unknown or Variable composition, Complex reaction products or Biological materials.

10 Organisation for Economic Co-operation and Development.

2.4 Chemical Safety Assessment and Chemical Safety Report

In accordance with Annex I of REACH, at production/import volumes of 10 or more tonnes per year, a Chemical Safety Assessment (CSA) documented in a Chemical Safety Report (CSR) is required. This includes a hazard assessment and, if the substance meets the criteria for classification and labelling or is assessed to be a PBT/vPvB[11] substance, an exposure assessment including the generation of exposure scenarios and finally a risk characterisation. A registrant may decide to develop exposure scenarios even if the substance does not meet the above mentioned criteria, in order to describe and implement how he controls the nanomaterial at his own site and recommend downstream users to control exposures to human health and the environment.

The behaviour and effects of nanomaterials are dependent on several characteristics, including size, number concentration, surface area, charge and overall surface reactivity. The risk assessment related to both human health and the environment has to take into account these characteristics. In order to address the specific hazards associated with nanomaterials, additional testing or information may be required. To determine specific hazards associated with nanomaterials, current test guidelines may need to be modified. Until revised and specific test guidelines for nanomaterials exist, toxicity testing will have to be carried out according to already existing guidelines and/or by corresponding test methods, as much as possible supplemented with nano-specific additions because these can provide very useful and relevant information (see section 3.2).

2.5 Exposure-related information

Aside from data to characterize the hazard of a substance, REACH also requires information on exposure, use and risk management measures to be provided upon registration. For substances manufactured or imported in quantities below 10 tonnes/year, however, the data requirements are very limited. For these substances the following information should be provided (Annex VI):

- Information on the tonnage; a brief description of the technological process(es) used in manufacture or production of articles; information on the form (substance, preparation or article) and/or physical state under which the substance is made available to downstream users; a brief general description of the identified use(s).
- Guidance on safe use, consistent with information in the safety data sheet on *e.g.* safe handling and exposure control/personal protection.
- Information on exposure for substances registered in quantities between 1 and 10 tonnes/year: the main use category (industrial, professional and/or consumer); whether use is in closed system, non-dispersive, dispersive and/or results in inclusion into or onto matrix; significant route(s) of exposure for humans and the environment; pattern of exposure (accidental/infrequent, occasional and/or continuous/frequent).

It is important to note that these limited requirements also hold for substances manufactured or imported in volumes over 10 tonnes/year when they are not classified as dangerous or not PBT/vPvB. The resulting information is more of a qualitative than quantitative nature, and will not likely provide details on the various sizes and shapes/forms of the nanomaterials to which humans and the environment are exposed in different exposure scenarios.

[11] PBT/vPvB: persistent, bioaccumulative and toxic or very persistent and very bioaccumulative.

Only for substances manufactured or imported in volumes over 10 tonnes/year that are classified as dangerous or PBT/vPvB, a more detailed exposure assessment is required, considering all life-cycle stages resulting from the manufacture and identified use(s). This assessment includes development of exposure scenarios (including description of operational conditions and risk management measures) and exposure estimation (Annex I). Question is, however, if monitoring data are available for nanomaterials or if existing exposure models like EUSES[12], ConsExpo[13] and EASE[14], are suitable for providing exposure estimates for nanomaterials.

[12] European Union System for the Evaluation of Substances; a quantitative risk assessment tool for chemicals.
[13] Software model to calculate consumer exposure.
[14] Estimation and Assessment of Substance Exposure; software model to calculate occupational (worker) exposure.

3 General risk assessment issues for nanomaterials

3.1 Introduction

In the past years, several analyses on the suitability of current risk assessment approaches for nanomaterials have been performed. These are carried out by various international scientific organizations and committees such as the EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR, 2006, 2007b, 2009), the Royal Society and Royal Academy of Engineering (2004), VDI Technologiezentrum GMbH (2004), Nanotechnology Research Co-ordination group (NRCG, 2006, 2007), Environmental Defense – Du Pont (2007), Nanotechnology Industries Association (Arnold, 2007), US Environmental Protection Agency (US EPA; 2008) and Defra (Crane and Handy, 2007).

Recently, an extensive summary of these analyses has been reported by Defra (Rocks et al., 2008). All the information available until May 2008 is clearly and comprehensively reviewed in this report. Furthermore, it also contains an evaluation of the suitability of the current OECD test guidelines for the testing of nanomaterials. A similar evaluation has been made by the OECD Working Party on Manufactured Nanomaterials (WPMN) (OECD, 2008a).

It is also to be noted that the OECD-WPMN has set up a Sponsorship Programme to provide guidance on the methods used to assess safety, and to derive valuable and relevant information on the safety of manufactured nanomaterials. This with the intention to improve the understanding of nanomaterials and, if possible, to understand what information may be generalized across different nanomaterials or classes of nanomaterials. In order to derive that information a list of ‘endpoints for testing nanomaterials’ has been formulated (see Appendix 2).

3.2 Suitability of current test guidelines for nanomaterials

For chemical risk assessment, a range of critical toxicity endpoints and associated test guidelines have been established by organizations and regulatory bodies worldwide (including OECD, US EPA, EU). In Appendix 3 a comprehensive overview of these test methods for physicochemical properties and human toxicity is presented together with their suitability to determine the toxicity of nanomaterials (copied from Rocks et al., 2008). A similar analysis of test guidelines was performed by the OECD-WPMN (OECD, 2008a). The testing methods can be split in three parts: physicochemical properties, human health effects, and environmental effects. A more detailed description of all tests can be found in the review report (Rocks et al., 2008). Here only the relevant issues with respect to the testing of nanomaterials, as identified in the review report by Rocks et al. (2008), are summarized. However, some of the issues mentioned are not specific for nanomaterials, but are also relevant for bulk substances.

3.2.1 Physicochemical tests

The physicochemical properties of a substance will, to some extent, determine the exposure route to be used in further toxicity studies. Within general risk assessment frameworks for non-nano (bulk)

materials, there is a suggested tiered approach to determine the physicochemical properties of a substance in order to eliminate unnecessary tests (reviewed by Rocks et al., 2008). In this testing scheme the particle size distribution, if appropriate, is determined at a later stage. For nanomaterials, however, it is suggested that the particle size distribution is already to be determined in an early stage, because it is thought to have a major impact on toxicity testing.

Other relevant issues identified in physicochemical tests (based on Rocks et al., 2008):

- Observation of material – Manufactured nanomaterials are generally assumed to be solid particles with at least one dimension less than 100 nm. The materials will not be observable by the human eye, and testers need a microscope to be able to observe the particles. With a number of the physicochemical tests, the endpoint is assumed to be observable by eye using the apparatus listed in the Testing Methods in Appendix 3.
- Amount of material – A number of methods require large amounts of material to be used in the test, which may pose a problem with nanomaterials in development.
- Use of appropriate controls – Any testing methods should be standardized using 'known' nanomaterials to ensure reproducibility.
- Appropriate tests – A number of tests are not appropriate for solid material (which most of the manufactured nanomaterials are). Also some properties can not be determined for certain nanomaterials such as melting and boiling point for metal oxides.
- Methods of analysis – There is uncertainty whether analytical techniques such as HPLC et cetera are suitable for nanomaterials with variable surface chemistry, solubility and reactivity. New techniques have to be developed and validated for each new variation, a long and laborious process. Electron microscopy (EM) can be used for determining the presence of nanomaterials; the composition can be analyzed by using Energy Dispersive X-ray (EDX) and X-ray photoelectron spectroscopy (XPS). For larger amounts, techniques such as X-ray Diffraction (XRD) and electron paramagnetic resonance spectroscopy (EPR) can be used to identify crystal structure.
- Characterisation of the specific surface area (SSA) – This can be done for a non-porous material by mathematical derivation from measurements obtained from electron micrographs. Porous nanomaterials will have an increased surface area which is more difficult to determine by electron microscopy, however the BET theory measuring the physical adsorption of gas molecules onto a solid surface may be suitable to determine the surface area of porous nanomaterials (Brunauer et al., 1938).

3.2.2 Human health toxicity tests

In general, toxicity testing methods as established for non-nanomaterials are considered suitable for the determination of the effects of nanomaterials on human health. However, there are a couple of concerns (based on Rocks et al., 2008):

- Mass concentration – Mass concentration (in mg/kg or mg/mL) may not be an appropriate metric for dosage of nanomaterials.
- Appropriate route of exposure – For initial *in vivo* toxicity testing methods normally the oral exposure route is used. However, for testing of nanomaterials, this may not be sufficient and administration via dermal or inhalation routes is likely to be more applicable. Furthermore, the effect of oral administration of nanomaterials on gut flora may show toxic effects which are not investigated and identified during routine toxicity testing (which also counts for bulk materials).
- Duration of tests – Sub-chronic or chronic studies are likely to be the most appropriate to study the toxic effects of nanomaterials since the duration of human exposure to small amounts of nanomaterials will be over a longer period of time. Single or short-term exposures are likely to

occur with high concentrations of nanomaterials as a result of accidental release. This point also holds for non-nanomaterials.

- Detection of nanomaterials – Whereas the potential toxic effects of nanomaterials will be detectable by using light microscopy, their presence, as single particles or in small aggregates, will not be. Therefore, to show the presence of nanomaterials within a histological sample it will be necessary to use EM, which may be very laborious and time consuming.
- Distinction and identification of nanomaterials – As the normal analytical detection methods may not be suitable to detect the presence of nanomaterials within a sample (see above), and EM techniques only show their presence, not their chemical structure, additional techniques such as EDX and XPS should be applied to elucidate the structure. This is essential for the identification of nanomaterials (both manufactured and naturally occurring).
- Systemic effects of toxicity – The most probable scenario is that a nanomaterial, after entering the body, will relocate in the organism and exert a systemic effect at a target site. This cannot be determined by single cell *in vitro* studies and therefore the need for animal experimentation remains until more developed screening tests are available or the relationship between the physicochemical properties of a nanomaterial and its toxic effect can be determined. Again this concern also holds for bulk materials.
- Effect of particulate number – Given the small particle sizes of nanomaterials and the normal dosimetrics in toxicity studies (mass concentration in mg/kg), there is a distinct possibility that due to the large amount of nanomaterial to be administered (which may no longer be representative for the actual exposure situation), toxic effects induced are a consequence of an overload phenomenon, rather than a consequence of exposure to the nanomaterial itself (or a combination of both).
- Solution or suspension of (nano)material – the distinction between a solution or suspension of a material, whether in nanoform or in bulk form, for use in sample preparation must be considered. However, it is likely that this will only be a problem with long term administration of the test substance as the suspension may precipitate out over time (sediment).
- Use of appropriate solvent – whilst the test nanomaterial may be soluble and stable in an organic solvent, the effects of the solvent on the test system must also be considered. Conversely, the potential of the nanomaterial to interact with the surrounding media (*e.g.* plastic of syringe, cell culture media) must also be considered in the administration of the nanomaterial. This concern also holds for bulk materials.

3.2.3 Ecotoxicity tests

The ecological information required under REACH of manufactured nanomaterials was considered in depth by Crane and Handy (2007).

The areas where the current ecotoxicological testing methodology was identified as not fit for purpose were:

- Relating macroscale to nanoscale – Current chemicals regulations (including REACH) do not distinguish between the nanoscale and macroscale forms of substances, so ecotoxicity tests performed on the macroscale form may, from a legal point of view, need to be accepted for both macroscale and nanoscale forms by regulatory authorities. This needs to change so that, at the very least, an evidence-based case is presented by manufacturers to show that there is no difference in the hazards of nanoscale and macroscale forms of the same substance. At present macroscale material toxicity cannot be related to nanoscale material toxicity, so currently evidence can only come from (rapid) testing;
- Exposure in test systems – Organisms in ecotoxicity tests should be exposed to nanomaterials in a way that is environmentally relevant. The homogenous dispersion currently recommended in

ecotoxicological testing may not reflect this. In the environment nanomaterials may react to their surroundings by agglomeration and aggregation after which precipitation is likely to occur, or they may react with other (naturally occurring) substances that may attach themselves to the surface of nanomaterials;

- Acute to chronic extrapolation – In most environmental risk assessment frameworks chronic toxicity is predicted from acute toxicity data by applying (large) assessment factors. For nanomaterials there is currently not enough empirical data (including data on bioaccumulation potential) to derive such assessment factors;
- Mass concentration is commonly used as a determinant of dose, but other metrics like for instance (combinations of) specific surface area, particle size, zeta potential, and shape might be better suited to quantify adverse effects across nanomaterials.
- Partition coefficient – There are some concerns about whether or not the partition coefficient test works for nanomaterials. This has implications for risk assessment strategies that use the partition coefficient as a trigger for requiring either sediment toxicity tests or bioaccumulation studies.

3.2.4 Additional OECD results

In the progress report of the OECD-WPMN (OECD, 2008a), all the OECD test guidelines were reviewed for applicability for nanomaterials. Only for physicochemical properties the tests were divided in 3 categories (I: applicable for nanomaterials, II: applicable for some nanomaterials or under some circumstances and III: not applicable for nanomaterials). The outcome of the evaluation (shortly summarized below) was generally similar to the one conducted in the review report by Rocks et al. (2008), with some slight deviations (*e.g.* on boiling point).

With respect to health effects, the general conclusion is that the OECD guidelines are appropriate for investigating the effects of nanomaterials with the important proviso that additional consideration needs to be given to the physicochemical characteristics of the materials tested, including such characteristics in the actual dosing solution (see section 3.2.2). However, in some cases there is need for further modification of the OECD guideline. This applies in particular to studies using the inhalation route and to toxicokinetic (ADME[15]) studies. Finally, it is important to build upon current knowledge and practical solutions in relation to *in vitro* test approaches.

More specifically:

1. The current test guideline on toxicokinetics is old (1984) and is currently being revised. This test guideline as well as the draft revised version only give very general guidance. The question is whether general modifications of this guideline for nanomaterials are sufficient, possibly specific studies are needed on a case-by-case basis. Absorption through cellular membranes is crucial. It is unknown which characteristics determine the absorption via the different exposure routes. Distribution, metabolism and excretion studies are also very important, especially the passage of nanoparticles through barriers like the blood-brain barrier and the placenta.
2. Current test guidelines for oral exposure are appropriate but the test endpoints may need to be extended, *e.g.* with cardiovascular effects. Test guidelines for inhalation exposure have recently been updated. One important aspect that has been changed in these revised versions is the inclusion of examination of the entire respiratory tract, which makes the tests more suited for assessment of nanomaterials. However, specific attention to the translocation of nanoparticles from the lung to blood and brain, as well as the dose metric relevant for nanomaterials are as yet not included in

[15] Absorption, Distribution, Metabolism and Excretion.

these revised guidelines. For all repeated dose studies, including those via the respiratory tract, more information on cardiovascular effects, immunological effects and inflammation is desirable. Furthermore, also endpoints like reproductive toxicity/embryotoxicity as well as genotoxicity/carcinogenicity are worthwhile to investigate.

3. It is yet unknown whether the currently used assays for determination of genotoxicity will be valid for nanomaterials. This is in particular the case for bacterial assays since mechanisms of genotoxicity of nanomaterials may differ from that of other chemicals.

With respect to environmental effects, the validity and appropriateness of existing testing methods for nanomaterials is often questioned. According to the OECD (2008a), there is a lack of standardized protocols for testing ecotoxicity; guidance on preparation, delivery measurement and metrology in the existing test guidelines is currently insufficient for testing nanomaterials. The interactions of nanomaterials with the environmental matrices need to be assessed (like aggregation, shielding of the surface of nanomaterials with (dissolved) humic acids in water, complexation with organic carbon in soil as well as with other soil constituents like clay minerals). Exposure and dose-effect models need to be adapted. There is a general need for guidance for sample preparation and dosimetry. On this latter issue, a draft document has recently been written by the OECD-WPMN, in which guidance for nanomaterials has been proposed (OECD, 2009).

3.3 Currently proposed risk assessment approaches for nanomaterials

Of the currently proposed risk assessment approaches for nanomaterials, the one proposed by SCENIHR (2007b) is the most extensive one. The starting point in their approach, which was developed for assessing the potential risks from engineered nanomaterials, is the adequately characterized nanomaterial. Both human and environmental risks from exposure to this nanomaterial are then to be identified in a process involving 4 different stages (see also Appendix 4):

Stage 1: to identify whether the manufacture, use and/or end of use disposal or recycling could result in exposure of humans or environmental species and ecosystems;

Stage 2: to characterize the nature, level and duration of any exposure;

Stage 3: to identify the hazardous properties of any form of the nanomaterial to which significant exposure is likely;

Stage 4: to assess the risk.

In steps 1 and 2 the focus is on exposure to the nanomaterial, and when no human or environmental exposure is expected or this exposure is expected to be very low, the process stops or the nanomaterial is considered a low priority for hazard assessment. When, however, there is a potential for exposure, then the assessment proceeds to stage 3, the hazard assessment stage. In step 3, the focus is first on carefully selected *in silico* and/or *in vitro* testing and then, when effects are observed, either limited *in vivo* testing when effects are very similar to those of the bulk chemical, or further more specialised *in vitro* testing followed by *in vivo* testing when effects are not very similar to those of the bulk chemical or this is unknown. SCENIHR, however, does not provide details on the kind of *in vitro* or *in vivo* testing to be performed.

In 2009, SCENIHR concluded that ‘this framework remains appropriate although a few further details can be added in the light of recent publications’. These details include relevant physicochemical properties (size and size distribution, specific surface area, stability in relevant media, surface

adsorption properties and water solubility, if necessary extended with considerations about photoactivation and potential to generate active oxygen) and possibilities for read-across. This latter focuses on broad properties (*e.g.* fibres, rods and tubes versus particles) that might help identifying a testing strategy, although it is recognized that there is insufficient information to identify opportunities for read-across based on the general chemical composition of nanomaterials (SCENIHR, 2009).

On the basis of information on production volume, release and exposure and on toxicological screening information, a hazard trigger algorithm as a potential prioritization tool for regulators has been proposed by Howard and De Jong (2004), reviewed in VDI Technologiezentrum GMbH (2004) (see Appendix 5). In this algorithm a first step is to focus on the larger production volume materials (> 1 tonne/year) as well as those for which aerosol release and/or direct exposure (consumers, workers and/or environment) can be expected. If neither of these conditions is met, or if the material is rapidly soluble in water, it is considered as a low priority material for risk assessment.
For all other materials the next step is to distinguish between fibres and particles. Fibres < 5 μm in length and particles > 100 nm in diameter are considered as intermediate priority materials for risk assessment. In the final step toxicity and ecotoxicity is screened for the larger fibres (length > 5 μm) and smaller particles (diameter < 100 nm). If no (eco)toxicity is expected they are considered as intermediate priority materials, but if either human or environmental toxicity (or both) is expected or unknown, the material is considered as a high priority material for risk assessment. In this final step, toxicological screening is on lung toxicity, systemic effects, oxidative stress, endocrine disruption and sensitising potential, and ecotoxicological screening is on persistence, long range transport and biomagnification. However, no details are provided on the kind of tests (*in vitro* and/or *in vivo*) that would provide such screening information, nor is specified what kind of studies should be performed to complete the risk assessment (VDI Technologiezentrum GMbH, 2004).

Another approach which is currently explored within the RIVM and which was originally proposed with EU partners as part of a 7th EU Framework Programme proposal (but did not make it), is the NAPIRA Framework. This proposal is based on tiered testing, using (standard) *in vitro* and *in vivo* testing methods with, if necessary, nano-specific modification. In the proposal, tier 1 testing consists of local toxicity tests as well as *in vitro* kinetics studies. Depending on the outcome of tier 1 the next tier comprises four options for further testing, based on the presence or absence of local effects and systemic availability.

Despite a lot of attention the last few years for nanomaterials, it can be concluded that at the moment there are only a limited number of risk assessment approaches described. And those that have been described, just broadly outline the framework, with first a focus on exposure and in later stages/steps, only when a potential for exposure has been identified, a focus on hazard, however without specifying exactly what kind of *in vitro* and/or *in vivo* testing is to be performed in order to provide the necessary (screening) information for nanomaterials.

4 Nanosilver as a case study for registration under REACH

The case study concerns a hypothetical registration of a substance (metallic silver) that exists in the nanoform (nanosilver, a first generation nanomaterial) as well as in the bulk form, with a total tonnage within the 10 – 100 tonnage band.
The nanoform, characterized by a spherical particle (no aggregates or agglomerates) with a size of 15 ± 5 nm, is used in a bathroom cleaning product, *i.e.* a trigger spray containing 1 % nanosilver particles.
Emission to the environment may occur during all life cycle steps. For consumers the use of the product may result in both dermal and inhalation exposure to nanosilver. Workers may also be exposed (during the production of nanosilver particles and the formulation of the cleaning product), but are not dealt with in this report.

As working hypothesis, it can be assumed that nanosilver forms dissolved free silver ions in aqueous solutions by dissolution and subsequent oxidation. Dissolution is a process that basically differs from the process of dissolving of chemicals. Chemicals that dissolve will become hydrated and will yield molecules that are surrounded by water molecules without losing their chemical integrity. Salts that dissolve in water generally yield hydrated cations and anions, *i.e.* charged ions surrounded by water molecules. Metal solids (*i.e.* metal not in salts) do not dissolve as such, independent of the metal solids being present in bulk form or in nanoform. Instead, oxidation of the metal will take place at the surface of the solids. This will yield oxidized metal ions (like Ag^{+}) that are released in the water compartment surrounding the (bulk or nano) metal solids. Similar to the case of metal salts, the metal ions thus formed will become hydrated and surrounded by water molecules. Metal solids are not distributed in the environment according to equilibrium-based processes and instead will deposit to either sediment or soil instantaneously. Deposited salts will distribute according to the fundamentals of equilibrium partitioning. As the chemical form (silver metal) of nanosilver initially is similar to bulk silver, the nano-specific physicochemical properties of nanosilver are to be compared to the physicochemical properties of silver metal. As dissolution of nanosilver yields dissolved silver ions, the environmental distribution of nanosilver is best compared to the distribution of silver salts. Influence of nano-specific characteristics on nanosilver toxicity are the sum of the contribution of the nanoparticles *sec* and the contribution of the silver ions released. As bulk silver solids are non-toxic, the kinetics of dissolution are of importance in this respect, resulting in the silver cation (as measure for dissolved silver species) being the determining factor for (systemic) toxicity. This means that in principle toxicity data on any silver compound, when expressed as the silver cation, can be used to determine the (systemic) toxicity of silver (cf. former Technical Guidance Document for environmental risk assessment for metals and metal compounds), with data on soluble silver salts probably more worst case for (nano)silver than data on less soluble/insoluble silver salts, provided that the anion does not significantly contribute to the toxicity of the silver salt.

In conformity with the requirements under REACH and based on the above-mentioned assumptions for the nanoform and on the dissolution to the ionic species, a CSR has been completed on the hazard assessment and exposure assessment parts (as far as possible and considered relevant for the purpose of this case study). This CSR can be found in Appendix 6. In this chapter, the information requirements as well as the conclusions on the available silver data are summarized.

Disclaimer: The data presented in the CSR on bulk silver should not be seen as a complete overview of the toxicity of this compound. No in depth literature search was performed, only readily accessible data

were used. The primary information source in this case was an assessment report on silver thiosulphate, drafted within the pesticide framework (CTGB, 2004). For nanosilver the primary information source was a review by Wijnhoven et al. (2009). This was supplemented with relevant information found on nanosilver upon searching the literature for studies that became available since submission of the review.

4.1 Substance identity and physicochemical properties

4.1.1 Information requirements and availability

For substance identity and physicochemical properties the data requirements under REACH and available information are given in Table 1.

Table 1. Information required under REACH and information available.

Information requirements up to 100 t/yr according to Annexes VII + VIII	Information available [a)]	
	Silver in nanoform	Silver in bulk form*
State of the substance at 20 °C and 101.3 kPa	√	√
Melting/freezing point	Read-across	√
Boiling point	Read-across	√
Relative density	Read-across**	√
Vapour pressure	Not relevant	Not relevant
Surface tension	Not relevant	Not relevant
Water solubility	√	√
Partition coefficient *n*-octanol/water	Not relevant	Not relevant
Flash-point	Not relevant	Not relevant
Flammability	Not relevant	Not relevant
Explosive properties	√	√
Self-ignition temperature	Not relevant	Not relevant
Oxidising properties	Not relevant	Not relevant
Granulometry	√	√

[a)] * information on metallic silver; ** caution is necessary (see section 4.1.2.2); √ information is available; Read-across: for nanosilver this information is assumed to be the same as for silver in bulk form; Not relevant: the endpoint given is by definition not of relevance for the risk assessment of either the nanoform or the bulk form of (metallic) silver.

4.1.2 Summary of available data for substance identity and physicochemical properties

4.1.2.1 Substance identity

For silver and nanosilver, the names and other identifiers (CAS number, IUPAC name, et cetera) are the same. The typical purity of nanosilver is > 99.99 %.

4.1.2.2 Physicochemical properties

Nanosilver is a solid under standard conditions. Specific data for melting/freezing point, boiling point and relative density are not available for nanosilver, but it can be assumed that these are similar to those of metallic bulk silver. Interpretation of relative density of nanosilver, however, needs caution. Very small nanosilver particles will have a density > 1, but will not precipitate due to upwards pressures in the water that might very well counteract gravitational forces. This may complicate the prediction of the exchange of nanosilver between water and sediment/soil and may ask for an additional parameter for risk assessment of nanoparticles, especially since this may also influence fate and behaviour in aquatic toxicity tests.

Although no studies on the explosive properties of nanosilver are present, in general dust explosions may occur when the particle diameter is smaller than 1 – 0.1 mm. The combustion rate increases with smaller particle sizes with an optimal combustion at particle diameters of approximately 10 – 15 µm (Eckhoff, 2003). With respect to granulometry it is obvious that nanosilver particles are much smaller than bulk silver particles. Other properties are not relevant for silver.

Metallic bulk silver is a solid, for which the relevant properties are available. Bulk silver is not explosive.

4.1.3 Discussion on substance identity and physicochemical properties

4.1.3.1 Substance identity

Chemical identity

Distinct Chemical Abstract Service (CAS) numbers for silver and nanosilver are not available. CAS numbers are assigned for unique chemical species, with a molecular formula and a unique structure but not for 'materials' per se. When different CAS numbers are to be assigned to nanoforms of a bulk substance, it needs to be decided what makes a nanoform different from the bulk, and one nanoform different from the other.

Composition of the substance

The nanosilver particles in the case study are produced in a narrow particle size range, 15 ± 5 nm. Effectively, this means that the size distribution is assumed to be more or less monodisperse. In reality, however, the particle size distribution may be more multidisperse than monodisperse, *i.e.* the mean particle size may still be 15 nm but the distribution is much wider. Since physical, chemical and toxicological properties of nanosilver particles may depend on particle size, it is important to know how to deal with particles outside the intended range of 10 – 20 nm. Are they to be considered as impurities, or as part(s) of the substance, making the substance possibly a multi-constituent substance? As a further complication, metallic bulk silver is not specifically defined/characterized, other than that it consists of particles > 100 nm. This may give difficulties in comparing measured properties of metallic silver with those of nanosilver. So, for a proper read-across between the two, both forms in principle need to be properly characterized.

4.1.3.2 Physicochemical properties

Overall, most basic physicochemical properties of nanosilver are similar or assumed to be similar to those of bulk silver. Exceptions are explosivity, granulometry, and water solubility. Nanosilver may have explosive properties which are solely due to the small particle size. Although obvious that

nanosilver particles are smaller than bulk silver particles, for a proper risk assessment other information on granulometry than solely particle size distribution may be essential. Parameters such as specific surface area, surface charge and shape of the particles (*e.g.* rods versus ball-shape particles) are important as well. This is in agreement with the opinion of SCENIHR (2009, with reference to the work of OECD and ISO), who identified the main physical and chemical properties with respect to nanomaterial safety (see Table 2). At the moment, it is not clear which properties are the key parameters to predict hazard and exposure, and therefore information on all properties should be provided. In the future when more information on nanomaterials becomes available it may be possible to make a selection of the most relevant parameters.

Various methods are available for measuring the parameters given in Table 2, like dynamic light scattering, electron microscopy, dynamic imaging (Nanosight), thermal optical transmission, et cetera. Silver is insoluble, but nanosilver might form dissolved free silver ions in aqueous solutions by dissolution and subsequent oxidation, or it may be dispersed in water following agitation. There is no information available on the kinetics of dissolution in dependence of nanosilver particle properties (such as (time-dependent shifts in) size distribution, shape) and properties of the medium (like pH, dissolved organic carbon, silver-complexing ions). This information is important, as also stated by SCENIHR (2009, see Table 2), especially for nanosilver because dissolution will yield highly toxic free silver ions. Free silver ions in water can relatively easily be measured by means of a silver selective electrode. Recently, a new approach is introduced to differentiate the effect of silver ions from silver nanoparticles by scavenging the silver ions with glycine (Navarro et al., 2008). Also, OECD guidance (OECD, 2001) is available for the determination of the rate and extent to which metals and sparingly soluble metal compounds can produce soluble available ionic and other metal-bearing species in aqueous media under a set of standard laboratory conditions representative of those generally occurring in the environment. In human toxicology studies distinguishing between nanosilver and silver ions may be much more difficult, because dissolution may take place within the organism, which complicates measuring this process (see section 4.3.4.3).

Table 2. Main physical and chemical properties with respect to nanomaterial safety as defined by SCENIHR (2009).

<table>
<tr><th>Physical properties</th><th>Chemical properties</th></tr>
<tr><td rowspan="8">Granulometry:
- size
- shape
- specific surface area
- aspect ratio
- agglomeration/aggregation state
- size distribution
- surface morphology/topography
- structure, including crystallinity and defect structure</td><td>Structural formula/molecular structure</td></tr>
<tr><td>Composition of nanomaterial (including degree of purity, known impurities or additives)</td></tr>
<tr><td>Phase identity</td></tr>
<tr><td>Surface chemistry (composition, charge, tension, reactive sites, physical structure, photocatalytic properties, zeta potential)</td></tr>
<tr><td rowspan="2">Hydrophilicity/lipophilicity*</td></tr>
<tr><td></td></tr>
<tr><td>Solubility</td><td></td></tr>
</table>

* Not relevant for nanosilver.

In all cases, the fate and effect properties of the nanosilver particles basically need to be compared to those of bulk silver and the properties of silver salts like silver nitrate. This is the only way to assess the

nano-specific properties of nanosilver as compared to properties of the bulk material and dissolved silver ions. In essence, one wants to compare the risk of nanoparticles to the risk posed by bulk silver (*i.e.* of size in all dimensions > 100 nm). As the ultimate fate of nanosilver is dissolution to yield silver ions, the risks of silver salts need to be included too. For a proper comparison, however, additional data are needed, such as information on rate and extent of dissolution (also in comparison to metallic bulk silver) and on stability of the nanoparticle.

On basis of the evaluation of substance identity and physicochemical properties, there are some essential information gaps for the substance to be registered (*i.e.* (nano)silver). As indicated in the introduction of this chapter, it is assumed for the present case that some of these data is available/given. For instance, nanosilver is characterized by a spherical particle (no aggregates or agglomerates) with a size of 15 ± 5 nm.

These general assumptions will be used in the following paragraphs to assess the relevance of the toxicity data and to assess the exposure of the various groups.

4.1.3.3 Additional data needed

As indicated in Table 2, several specific properties for nanomaterials are needed to properly assess the risk. For the presented case of nanosilver (spherical particles of 15 ± 5 nm) some of these properties are known (*e.g.* size, shape, agglomeration/aggregation state), but there are also still some uncertainties. For the REACH requirements, however, only two main issues of uncertainty remain, which require additional information.

1. Nanosilver is assumed to be insoluble, but it might form dissolved free silver ions. As no information on the kinetics of dissolution is available, additional data on this kinetics is needed.
2. For fate estimations partition coefficients (*i.e.* K_p values) are needed, especially for the partitioning between sediment and water and for soil and water. At present specific values for nanosilver are not available.

4.2 Environmental hazard and exposure assessment

4.2.1 Information requirements and availability

For the environment the data requirements under REACH and information available are given in Table 3.

Table 3. Information required under REACH and information available for environment.

Information requirements up to 100 t/yr according to Annexes VII + VIII		Information available*	
		Silver in nanoform	Silver in bulk form
Adsorption/desorption	Screening test	X	√
(Bio)degradation	Ready biodegradability Hydrolysis as a function of pH	Not relevant Not relevant	Not relevant Not relevant
Bioaccumulation	Fish	X	√
Acute toxicity	Invertebrates (preferably *Daphnia*) Fish	√(*Daphnia, Ceriodaphnia*) √	√ √
Growth inhibition aquatic plants (preferably algae)		√	√
Activated sludge respiration inhibition test		X	X

* Information on silver in bulk form is information on silver salts; √: available; X: not available; Not relevant: the endpoint given is by definition not of relevance for the risk assessment of either the nanoform or the bulk form of silver (salts).

4.2.2 Summary of available hazard data for environment

4.2.2.1 Fate and behaviour

There is no information available on the environmental distribution of nanosilver, although as an element silver cannot be degraded and therefore degradation of silver and nanosilver is not applicable. The general colloidal properties of nanoparticles as well as information on the behaviour of silver ions in the environment indicate that adsorption to sediment and soil particles, and complexation with dissolved organic carbon (DOC) and particulate matter (algae) are important processes, because silver is known to bind strongly to oxygen- and especially sulphur-containing phases. Other relevant processes may be aggregation (leading to sedimentation of nanosilver particles) and stabilization in the water column by organic material. A potentially important dissipation pathway for nanosilver particles is dissolution and subsequent oxidation to yield ionic silver. This process may be significant especially for nanosilver because free silver ions are highly toxic. Studies with nanosilver on sorption to soil, suspended matter and sediment and information on bioaccumulation are lacking. The determination of this data for nanosilver particles and nanomaterials in general seems of high relevance since differences are expected with respect to the bulk form based on differences in physicochemical properties (see section 4.1.3.2).

For silver salts, data on sorption to soil, suspended matter and sediment and information on bioaccumulation are available, but studies with metallic silver are lacking, although it can be argued that metallic silver as such is not mobile.

4.2.2.2 Ecotoxicity data

Ecotoxicity of nanosilver was determined in short-term studies with fish (adults and juveniles), invertebrates (adults and neonates), and algae. LC_{50} and EC_{50} values[16] determined in standard toxicity

[16] Concentration at which mortality is 50 % (LC_{50}) or at which 50 % of the test population is showing an effect (EC_{50}).

tests ranged from 0.04 mg Ag/L for invertebrates (*Daphnia pulex* adults) to 7.2 mg Ag/L for fish (*Danio rerio* juveniles). No long-term NOEC[17] values are available.

Toxicity of silver salts was studied in short-term and long-term tests with fish and invertebrates (adults and juveniles/neonates) and algae. Effect concentrations ranged from 0.0034 mg Ag/L for algae (EC_{50} growth rate of *Pseudokirchneriella subcapitata*) to > 10 mg Ag/L for fish (LC_{50} for *Pimephales promelas*, juveniles). Chronic NOEC values ranged from 0.00024 mg Ag/L for fish (*Oncorhynchus mykiss*, adults) to 0.0016 mg Ag/L for invertebrates (*Daphnia magna*, adults).

4.2.3 Summary of environmental exposure assessment

Under REACH environmental exposure assessment is only necessary if the substance is classified as being dangerous for the environment or is considered to be a PBT/vPvB substance. This is the case for nanosilver and an exposure scenario should be developed.

In principle it is possible to compare (measured) exposure levels with established safe levels of nanosilver in the environmental compartments (so-called PNEC[18] derivation). However, under REACH environmental concentrations are predicted with the model EUSES (or similar models), which uses many QSPR[19]-calculations as input, mainly based on physicochemical properties (*e.g.* K_{OW}) of the substance, to come up with an estimate for the environmental distribution. This limits the usefulness of EUSES for metals, although when measured partition coefficients (K_p values) are available the calculation of these K_p values (usually based on log K_{OW}, which is not applicable for metals) can be avoided and an environmental distribution estimated. For nanosilver, however, these K_p values are not (yet) available, which renders this approach impossible.

Alternatively in the past a worst-case approach has been used in which it is assumed that 100% of the substance ends up in surface water as metal ions. This approach was also applied for nanosilver, resulting in a PEC_{local}[20] being much higher than the PNEC and thus indicating a potential risk. Dissolution of (nano)silver, however, is a slow process and considering the low water solubility of nanosilver, it can be questioned whether the estimated emission scenario is realistic. It can be expected that nanosilver particles precipitate quickly to the sediment, where the rate of dissolution is much slower. In addition, in algal tests it was shown that the observed toxicity was the result of both Ag^+ ions and suspended particles of nanosilver (Navarro et al., 2008). This suggests that despite their low solubility nanosilver particles are biologically available and can exert toxic effects, which further undermines the reality of the estimated exposure scenario (although not necessarily the risk potential).

4.2.4 Discussion on fate and behaviour and ecotoxicity

4.2.4.1 Hazard

REACH requires that the available hazard data on a substance to be registered are adequate with respect to:

– the data requirements (as specified for the tonnage band applicable to the substance);

[17] No Observed Effect Concentration: the highest concentration at which no effect is observed.
[18] Predicted No Effect Concentration: the highest concentration for which it is predicted that no effect will be observed.
[19] Quantitative Structure Property Relationship.
[20] Predicted Environmental Concentration close to the point of release.

- classification and labelling;
- derivation of PNECs.

With respect to nanosilver it can be concluded that:

- only acute aquatic toxicity tests are available;
- information on adsorption/desorption kinetics is lacking;
- an activated sludge respiration inhibition test is missing;
- the available data are sufficient to determine classification and labelling for the environment (N; R50-R53, S60, S61);
- the available data are in accordance with OECD guidelines and allow the establishment of PNEC(s) to be used in risk characterisation. However, it is not clear if the dose metric normally used for substances in bulk form (*i.e.* based on mass) is also relevant for nanomaterials (here numbers and surface area are probably more appropriate).

For the bulk form of silver it can be concluded that the available data:

- do not fulfil the data requirements for substances within the 10 – 100 tonnage band, as an activated sludge respiration inhibition test is missing. The key studies available are in accordance with recent (updated) OECD guidelines and allow the establishment of PNEC(s) to be used in risk characterisation (this has only been done for illustrative purposes for silver nitrate in the aquatic environment);
- includes a bioconcentration study in fish which is not required at this tonnage band;
- are suitable to conclude on classification and labelling. Based on the acute aquatic toxicity results (LC/EC_{50} < 100 mg/L), the classification and labelling for this substance should be N; R50-53, S60, S61.

Exposure-based waiving is not possible for the data requirements at this tonnage level. Also, exposure of the environment to nanosilver is expected due to the application of the substance in a household spray.

4.2.4.2 Fate and behaviour

There is no information available on the environmental distribution of nanosilver and relevant processes. A comparison with bulk silver can therefore not be made. Firstly, data on dissolution kinetics in dependence of nanosilver particle properties (such as (time-dependent shifts in) size distribution, shape) and properties of the medium (like pH, dissolved organic carbon, silver-complexing ions) are needed. Also quantitative information on aggregation and complexation of nanosilver particles is required.

Because of its colloidal properties, it can be expected that nanosilver will bind strongly to complexing and sorbing agents present in soil, suspended matter or sediment. Distribution coefficients (K_d values[21]) may differ between nanosilver and silver ions (sorption is not a relevant process for metallic silver in bulk form). Registrants are therefore requested to provide information on sorption and desorption of nanosilver in the aquatic and terrestrial compartment in dependence of particle properties (*e.g.* size and

[21] K_d is the distribution coefficient of a substance (in its environmentally relevant form, *e.g.* as ion) over two compartments, which highly depends on environmental conditions and the presence of counterions (among other factors). In fate models the K_d is often replaced by the partitioning coefficient K_p, which describes the partitioning of a substance (in its neutral form) over two compartments in equilibrium.

surface charge) and properties of the solid and liquid phases. Registrants should clarify if K_d values for silver ions can be used as (worst-case) estimate for assessing nanosilver concentrations in the aqueous phase, which is assumed to be the bioavailable fraction (although in algal tests it was shown that nanosilver particles themselves also contribute to toxicity). In principle, standardized OECD and US-EPA guidelines are available for quantifying sorption, although some adjustments might be considered necessary for the evaluation of nanomaterials (OECD, 2008a).

4.2.4.3 Ecotoxicity

Short-term tests with fish juveniles and invertebrates (adults and neonates) showed comparable results for nanosilver particles and silver salts. However, short-term LC_{50} values for adult fish indicate that toxicity of nanosilver particles was 100 – 1000 times less toxic than silver salts. A similar result was found for algae, where nanosilver particles are 50 times less toxic than silver salts. It is not clear if the results obtained for silver salts may also be used for nanosilver particles but it is expected that toxicity increases when more silver ions become available over time in solution. For this reason, aquatic toxicity tests, and especially long-term tests, should not be waived, unless the registrant can prove that nanosilver particles are less toxic than dissolved silver or that tests with silver salts (*e.g.* $AgNO_3$) can be considered as a worst case. Further, it is not certain if standard toxicity studies focus on the relevant endpoints for nanomaterials and if other test organisms should be considered as well (although this also holds to some extent for non-nanomaterials).

4.2.5 Additional information needs

Overall, the primary information needs regarding environmental hazard and exposure assessment deal with increasing understanding of the fate and behaviour of nanosilver in aquatic and terrestrial ecosystems, both with regard to exposure in laboratory test systems as with regard to exposure in a field setting. The major information in this respect deals with the kinetics of dissolution as impacted by the physicochemical composition of the exposure medium, and the complexation by (dissolved) organic material of particles of nanosilver. As it is expected that dissolution of nanosilver is a slow process, long-term testing appears to be necessary. In addition, tests that include both sediment and water phases (*e.g.* OECD Test Guideline 308) may shed light on the distribution of nanosilver over different environmental compartments.

With regard to toxicity, data are needed to allow for distinguishing between the toxicity of the nanoform and the toxicity of free silver ions. Also for toxicity chronic tests appear to be necessary to properly identify the risks of nanosilver, although the specific setup should be well considered. In flow-through and semi-static test systems the slow dissolution process of nanosilver may be missed due to refreshment of the water phase (although static test systems are also less suitable for chronic testing, especially for compounds that have low water solubility).

Finally, information needs to be generated on the best metrics to quantify toxicity.

At present for nanosilver there is no information available on:

- long-term toxicity in fish or *Daphnia*. This information is only required at the level above 100 tonnes/year, but considering that some nano-specific processes that lead to toxicity (*e.g.* dissolution of nanoparticles) take longer periods of time long-term toxicity studies are highly recommended;
- effects on microbiological activity in sewage treatment plants. This information is required at the tonnage band of 10 – 100 tonnes/year (this information is also lacking for bulk silver);

– toxicity in sediment. This information is only required at the level above 100 tonnes/year, but water-sediment tests may give information on partitioning as well.

4.3 Human health hazard and exposure assessment

4.3.1 Information requirements and availability

For human health hazard assessment the data requirements under REACH and the information available are given in Table 4. Please see chapter 5 of the CSR for study summaries (Appendix 6).

Table 4. Information required under REACH and information available for human health.

Information requirements up to 100 t/yr according to Annexes VII + VIII		Information available*	
		Silver in nanoform	**Silver in bulk form**
Toxicokinetics	When available	√	√
Acute toxicity	At least two routes	X	√ (oral, dermal)
Irritation	Skin, eye	X	√
Sensitisation	Skin	X	√
Repeated dose toxicity	At least 28-day study	√ (28-d rat oral, 28-d rat inhalation, 90-d rat inhalation)	√ (90-d rat oral, 90-d dog oral, 28-d rat dermal)
Mutagenicity	*In vitro* gene mutation bacteria	X	√
	In vitro cytogenicity mammalian cells	√ (*in vivo* micronucleus)	√
	(*In vitro* gene mutation mammalian cells)	X	√ + *in vivo* UDS[22]
Reproductive toxicity	Screening study	X	√ (Two-generation rat oral, developmental toxicity rat oral)

* Information on silver in bulk form is mainly on silver thiosulphate (CTGB, 2004), plus some published information on silver/silver compounds; √: available; X: not available.

[22] *In vivo* unscheduled DNA synthesis (UDS) assay.

4.3.2 Summary of available hazard data

4.3.2.1 Toxicokinetics

For nanosilver data are available from a distribution study following single exposure to ultrafine elemental silver particles (mean diameter 17 nm) via inhalation for 6 hrs, as well as following single intratracheal exposure to agglomerated ultrafine elemental silver particles (mostly larger than 100 nm, but also ultrafine particles present). For comparison reasons, silver nitrate was also instilled intratracheally. These data show that (nano)silver becomes systemically available following single inhalation and intratracheal exposure, possibly in part by oral absorption and by neuronal absorption; the latter because after inhalation silver is found in brain tissue, both in the olfactory and non-olfactory part. However, the form in which it is absorbed and present in tissues (as nanosilver particles, silver ions or both) is not clear, because measurements were on Ag. The clearance rate of silver from the lungs after inhalation exposure to nanosilver particles is much faster than after intratracheal exposure to agglomerated nanosilver, a part of which remained undissolved in alveolar macrophages for at least 7 days following instillation. Instilled water-soluble silver nitrate was also cleared rapidly from the lungs (supporting the theory of rapid solubilization of ultrafine elemental silver in the lung), albeit less rapidly than nanosilver. A comparison of tissue distribution was not possible, since following instillation data on Ag content in tissues other than lung and liver were not reported. Metabolism, protein binding and excretion following nanosilver administration were not investigated.

Systemic availability of (nano)silver is also demonstrated in two 28-day rat toxicity studies, with oral and inhalation administration of nanosilver (mean particle size 60 and 12 – 15 nm, respectively), as well as in one 90-day rat inhalation toxicity study (mean size of nanosilver particles 18 – 19 nm). Both exposure routes led to the presence of silver in (amongst others) lungs and brain. Following inhalation exposure, silver was also found in the olfactory bulb, in concentrations higher than in the brain. In humans, topical administration of nanosilver (mean diameter 15 nm) on damaged skin also resulted in absorption, as evidenced by measurable silver concentrations in blood and urine and signs of greyish discoloration.

As to the bulk form, it is known from available data on silver/silver compounds that silver is absorbed following oral, dermal and inhalation administration. It can be assumed that all silver compounds (including metallic silver) are transformed (at least in part) to the ionic species, and that internal exposure is to the silver cation (as measure for dissolved silver species). Absorbed silver binds to plasma proteins and as such is distributed across the body. An important excretion route for silver is via bile. Accumulation of silver can occur in various tissues, *e.g.* skin and eyes, in humans leading to argyria (blue/grey discoloration).

4.3.2.2 Toxicology

For nanosilver, data for repeated dose toxicity were available (for the oral and inhalation route) and an *in vivo* micronucleus test. Oral and inhalatory administration of nanosilver (with mean particle sizes of 60 and 12 – 15 nm, respectively, and doses up to 1000 mg/kg bw/day and $1.32 \cdot 10^6$ particles/cm^3, respectively) to rats for 28 days, as well as inhalatory administration for 90 days (mean particle size of 18 – 19 nm and doses up to $2.85 \cdot 10^6$ particles/cm^3), resulted in the presence of silver in various organs such as liver, lungs, brain and (following inhalation) olfactory bulb. Since measurements were on Ag, it is not clear whether silver is present in these tissues as nanosilver particles, silver ions or both. No histological changes were observed in the lungs following 28 days of nanosilver administration. However, when rats were inhalatory exposed to nanosilver for 90 days, lung function changes along with inflammation were seen. In the latter study the number of particles in the high dose was

approximately twice that in the high dose of the 28-day study, but the calculated surface area of nanoparticles in the highest dose was almost 5-fold greater than that in the high dose of the 28 days study. As to other organs than lungs, except for some liver damage, no other organ changes were observed in the two 28-day studies and in the 90-day inhalation study. However, it is to be noted that the histological examination of brain, respiratory tract and nervous system was not very extensive, nor did the studies include investigation of neurotoxicity parameters. Clinical effects were monitored, but were absent. No effects were observed in an *in vivo* micronucleus test carried out at the end of a 28-day oral study. Next to these *in vivo* studies, several *in vitro* studies with nanosilver have been described, displaying possible cytotoxic and immunotoxic effects of nanosilver.

With respect to the toxicity of silver in bulk form, it is known that silver compounds are relatively non-toxic, and that the main effect is accumulation of silver in various organs, *e.g.* skin and eyes. In humans the latter results in argyria, in most cases without accompanying health problems. In animal studies with silver thiosulphate (STS), the main effect was visible pigmentation in the gastrointestinal tract, liver and kidneys. This also points to (undesirable) silver accumulation. Except for this pigmentation, no other histological changes were observed in any tissue, including brain. Clinical effects were absent, as well as effects on neurotoxicological parameters (studied in a 90-day oral study with rats).

4.3.3 Summary of consumer exposure assessment

Since nanosilver is produced/used in total over 10 tonnes/year (see assumptions in introduction of chapter 4) and the hazard assessment indicated that both the bulk form and the nanoform are dangerous to the environment (see section 4.2.4.1), an exposure assessment as required under REACH was performed (see chapter 9 of the CSR). For the purpose of this report, this was only carried out for the nanoform and only for consumers, not for workers.

The exposure of consumers, as assumed to be dermally and inhalatory exposed to nanosilver via the use of a bathroom cleaning product, has been estimated using two different models (see chapter 9 of the CSR), one being the ECETOC TRA[23] (revised first tier version for REACH), the other ConsExpo (version 4.1).

The ECETOC TRA is a first tier model, based on default algorithms and default assumptions for input parameters. These default assumptions are fixed for most input parameters; only those for weight fraction and body weight (adult or child) can be replaced by product-specific values. The resulting exposure estimate is a worst-case estimate, because for inhalation this model assumes instantaneous release of the substance and no removal by *e.g.* ventilation, and for dermal exposure that 100 % of the substance is in contact with the skin.

For nanosilver and the assumed exposure scenario, the worst case estimates (per event) are as follows:

dermal exposure = 1.43 mg/kg bw

inhalation exposure = 0.0175 mg/m^3, corresponding to 0.0016 mg/kg bw

total exposure = 1.43 mg/kg bw

ConsExpo is a higher tier model, with more complex algorithms and more specific descriptions of exposure processes, with consideration of time-dependent processes (such as migrations/release from a matrix) and disappearance from a medium (such as via ventilation). The resulting exposure estimate is

[23] European Centre for Ecotoxicology and Toxicology of Chemicals, Targeted Risk Assessment.

therefore more realistic, also because most default input parameters can be replaced by more specific values.

For nanosilver and the assumed exposure scenario, the exposure estimates (per event) from ConsExpo are as follows:

dermal exposure = 0.0106 mg/kg bw

inhalation exposure = 0.000356 mg/m^3, corresponding to 3.3 x 10^{-6} mg/kg bw

oral exposure = 0.000317 mg/kg bw (= part of inhalation dose that is ingested)

total exposure = 0.011 mg/kg bw.

4.3.4 Discussion human health

4.3.4.1 Hazard

REACH requires that the available hazard data on a substance to be registered are adequate with respect to:

- the data requirements (as specified for the tonnage band applicable to the substance);
- classification and labelling;
- measure of dose-response.

For the bulk form of silver it can be concluded that the available data:

- amply fulfil the data requirements for substances within the 10 – 100 tonnage band. Key studies are in accordance with recent (updated) OECD guidelines, and no information gaps have been identified;
- are suitable to conclude on classification and labelling. No classification and labelling is necessary for human health;
- are suitable to provide a measure of dose-response. The data allow the establishment of NOAELs[24] that form the basis for the derivation of DNELs[25] to be used in risk characterisation. NB: this has not been done because it is outside the scope of this report.

When looking at the data on nanosilver, the situation is a bit different. It can be concluded that:

- for a nanomaterial, there is quite some information available;
- there is even data on the kinetics of nanosilver (after inhalation/intratracheal administration), where under REACH this is not a standard information requirement (yet desirable);
- the form of nanosilver tested in the available inhalation studies (non-agglomerated, spherical, mean particle sizes between 12 – 19 nm) seems representative for the nanosilver to be registered;

However, it is also true that:

- data on irritation/corrosion are missing;
- data on acute toxicity, sensitisation and reproductive toxicity are missing;
- data on mutagenicity are limited;

[24] No Observed Adverse Effect Level: the highest dose level at which no adverse effects are observed.
[25] Derived No Effect Level: the highest dose level at which no adverse effects are expected to occur.

- the nanosilver tested in the available oral study (average particle size 60 nm; shape/form unknown) may not be representative for the nanosilver to be registered;
- the available data are too limited to determine classification and labelling for human health;
- the available data may potentially provide a measure of dose-response, but how to express this measure and how it relates to the dose metric normally used for the bulk form is uncertain.

So, the data on nanosilver alone would not fulfil the data requirements for substances within the 10 – 100 tonnage band, given also that the repeated dose toxicity studies that are available were not supplemented with nano-specific additions such as for example those suggested by OECD-WPMN in their progress report (see also section 3.2.4). For the repeated dose testing of nanomaterials, the OECD-WPMN suggests the use of OECD guidelines that have enhanced ability to detect neurotoxic and immunotoxic effects and effects on the reproductive and endocrine systems, as well as enhanced pathology of the entire respiratory tract (when the inhalation route is studied), supplemented if necessary with investigation into specific adverse effects (*e.g.* on cardiovascular system).

Effectively, this would mean that there are information gaps for nanosilver, *unless* it can be demonstrated that relevant human exposure can be excluded or if not, that data on the bulk form of silver can be used to fill these gaps. For nanosilver, the first option is not valid (see also section 4.3.3), and the second option would only be valid if no difference is to be expected in intrinsic properties or classification and labelling. What can be concluded on that, given the data available?

When comparing the nanoform of silver to the bulk form (silver/silver compounds), it is clear that for both forms administration via the oral, dermal and inhalation route results in absorption of silver and distribution throughout the body. For the bulk form, internal exposure will be to the silver ion. However, for the nanoform, where available measurements were on Ag, it is not clear if it is absorbed and present in tissues as nanosilver particles, silver ions or both. But in view of its slow dissolution properties, it is likely to be nanosilver. For the nanoform also data on metabolism and excretion are lacking. Hence, the available data do not allow a conclusion on the kinetics of the nanoform being comparable or different to that of the bulk form.

When comparing the toxicity data for nanosilver to those of the bulk form, it is clear that for both forms repeated administration did not result in clinical effects or other marked toxic effects, despite the presence of silver in various tissues, such as liver, lungs and brain. For the bulk form, the silver concentrations in several tissues were such that they resulted in histologically visible pigmentation. That was not the case for the nanoform, for which it is not clear if it is present in tissues as nanosilver particles, silver ions or both. Furthermore, although there are indications that exposure to nanosilver can lead to damage to the lungs (after inhalation) and to measurable concentrations in the brain (including the olfactory part), neither brain and respiratory tract nor nervous system have been extensively examined histologically. Also no neurotoxicity parameters (*e.g.* a functional observational battery) have been investigated, whereas for the bulk form this was done and appeared not to be affected.

When comparing the *in vitro* data on nanosilver and the bulk form, it seems that both may have cytotoxic and immunotoxic effects. A real comparison is however not possible, since none of the investigated parameters have been tested for both compounds in the same study.

Hence, the available data do not allow a conclusion on the toxicity of the nanoform being comparable or different to that of the bulk form.

Unfortunately, therefore, no firm conclusions on the 'sameness' of the nanoform and the bulk form of silver can be drawn, making it uncertain whether use of toxicological data on bulk silver to fill data gaps for nanosilver is justified, and whether the one substance – one registration principle applies.

4.3.4.2 Exposure

The exposure estimates generated for nanosilver with ECETOC TRA and ConsExpo should be interpreted with caution, because both models are generally used for human exposure assessment of normal, non-nanosubstances. In this particular case of exposure to nanosilver via inhalation, ConsExpo has calculated the distribution of aerosols in a room from a (trigger)spray. However, this spray model (and its corresponding assumptions such as mass generation rate, airborne fraction, weight fraction non-volatile, density non-volatile, particle distribution median, particle distribution coefficient of variation) has not been validated for nanoparticles. For example, nanoparticles may stay longer airborne. Another assumption in ConsExpo is that particles smaller than 15 μm can be inhaled and are subsequently completely absorbed. For normal substances this is a worst-case assumption. Whether that is also the case for nanoparticles needs to verified, because especially for nanoparticles the size-dependent deposition and absorption is an important issue. This and other possible limitations of current exposure models are addressed further in section 5.4.3.

4.3.4.3 Additional data needed?

Although it is no standard requirement under REACH, as a first tier it would for nanomaterials in general be desirable to have data on kinetics, first of all to determine whether or not the nanomaterial can be transported across the portals of entry. Because if not transported across, the focus for further investigations may be limited to the potential local toxicity of the nanomaterial. But if the nanomaterial can become systemically available, then kinetic studies can be of help in determining in what entity (or entities) it becomes systemically available, and how it is distributed in the body. This is essential information in order to conclude on the toxic entity (or entities) of the nanomaterial and to direct further (toxicological) investigations at the right target organs at higher tiers.

For the specific case of nanosilver, it is already known that it becomes systemically available and that it exerts effects, based on the relatively abundant amount of information present. What is not known, however, is the form (as nanosilver particle, as free silver ion released from the nanosilver particle, or as both) in which it enters and is distributed in the body and exerts its effects, nor how that is influenced by different exposure routes or by different sizes and shapes of nanosilver. This is very important to know, because if internal exposure would be only to free silver ions, then the toxicological data available for the bulk form could be used for the registration of nanosilver (aside from the dosimetry issue). It is realized though, that there will most likely be a 'turning point' in the nano-characteristic behaviour of nanosilver, like for any other nanomaterial: above this point it will behave like the bulk material, below this point the nano-characteristics will come into play. This means that for certain shapes and sizes of nanosilver 100 % internal exposure to Ag^+ may be realistic because from that size/shape it behaves like silver in bulk form, whereas for nanosilver of smaller size or different shape the presence of nanosilver particles next to the presence of Ag^+ may be expected. In that case, the nanosilver particle is to be seen as a new chemical entity for which additional research is necessary, *e.g.* into interactions on cellular level and into passage over specific barriers or via specific transport (blood–brain, blood–testes, placenta, mother's milk, neuronal transport).

With respect to the above, two problems have been identified for the specific case of nanosilver:

1. in the studies available the methods used measure only Ag;
2. the turning point is not known.

The first issue is a general problem, *i.e.* current analytical methods for the most part only measure Ag, and thus do not differentiate between Ag^+ and nanoparticles. Of course, the presence of nanoparticles in tissues can be determined by *e.g.* electron microscopy, but as this is a very expensive and time-consuming technique, it is not for large scale use. Also a few methods for measuring Ag^+ are available

(for instance via an Ion Selective Electrode), but as this is difficult in contaminated, protein rich media, they need further development. One way might be by complexing Ag^+ with cysteine, as was recently done in a study with algae in order to disentangle the contribution of silver nanoparticles to the overall toxicity from that of Ag^+ (Navarro et al., 2008). So, for the measurement of both silver ions and nanosilver particles in different media analytical methods need to be (further) developed, taking into account determinants for silver ion generation such as pH and solubility characteristics.

The second problem identified raises uncertainty on whether the results of toxicological studies with other shapes/sizes of nanosilver than defined for our case (like for instance the oral repeated dose study with average nanosilver particles of 60 nm) can be used for or extrapolated to our case. Or, the other way around, whether toxicological studies with spherical, 12 – 19 nm nanosilver particles can be used for/extrapolated to differently shaped/sized nanosilver. So, more information is necessary on the absorption and distribution of different sizes and shapes of nanosilver in order to determine the turning point for nanosilver. At the moment, there are some very preliminary indications that this turning point for tissue distribution may be situated between 80 and 140 nm: in a pilot study in which rats received a single intravenous dose of nanosilver with particle sizes of 20, 80 or 140 nm (Lankveld and De Jong; personal communication), the tissue distribution was qualitatively similar for the different particle sizes (with the highest Ag concentrations in liver, spleen and kidneys), with the possible exception of the brain. For the largest particle size of 140 nm, in most brain samples no Ag was detectable, whereas for the nanoparticles ≤ 80 nm in most brain samples Ag was detectable. The turning point (nanosilver is present not only as Ag^+ but also as nanoparticles) is therefore expected to be somewhere between 80 and 140 nm, however, this is a very broad range. Currently, a definitive study is undertaken, in which a possible difference in behaviour of nanosilver of different particle sizes is to be confirmed, and in which the range for the turning point hopefully can be further narrowed.

As already concluded above, for the specific case of nanosilver the first tier/stages as regards the systemic effects have already been passed. Since there is also a (rough) indication of the degree of consumer exposure, a 'quick and dirty' risk assessment can be done in order to get an idea of the margins between this exposure and the doses in the toxicity studies with nanosilver at which effects were observed. In case these margins would be very high, this could perhaps be used as an argument to waive further testing for nanosilver for systemic effects.

Comparing the total exposure estimate from ConsExpo (0.011 mg/kg bw) with the doses tested in the 28-day oral toxicity study (30 – 1000 mg/kg bw; all assumed to be effect doses), the margin ranges from 2700 to 90,000. When comparing the inhalation exposure estimate from ConsExpo (0.000356 mg/m^3, for 25 min/day) with the doses tested in the 28-day and 90-day inhalation toxicity studies (0.48 – 61.24 $\mu g/m^3$ and 48.94 – 514.78 $\mu g/m^3$, respectively, for 6 h/day; all assumed to be effect doses), the margins range from 1.3 to 170 and 140 to 1400, respectively. When taking into account the uncertainties in both the exposure estimates and the toxicological data (among others with respect to dosimetry), it can be concluded that these margins are not of such a magnitude that they would support waiving of further testing for nanosilver for systemic effects.

All in all, when looking at all the available information and what can be inferred from that, additional information needs under REACH for nanosilver in first instance would be:

- data on the kinetics (including rate and extent) of dissolution to Ag^+ (see section 4.1.3.3), in order to decide on the sameness of nanosilver and bulk silver with respect to systemic effects;
- when not the same, data on transplacental passage/developmental toxicity (and when triggers are found, data on reproductive performance and possibly on genotoxicity for germ cells);
- data on *in vitro* genotoxicity, *i.e.* on the possibility to induce gene mutations. Given the antimicrobial properties of nanosilver, this should not be a test in bacteria, but in mammalian cells.

When positive, further genotoxicity testing, *e.g.* on the potential to induce local genotoxicity in the respiratory tract.

Data on acute toxicity and on irritation and sensitisation, although lacking and required at the 10 – 100 tonnage band, can be waived: they are not considered necessary in the case of nanosilver, because the available repeated dose toxicity studies on nanosilver do not provide indications for these effects. Since in these repeated dose toxicity studies also the reproductive organs were monitored and examined histologically, without adverse findings, these studies can be used as screening for fertility effects. That part of the reproductive toxicity testing can thus in first instance be waived.

5 Lessons learned from the case study for nanomaterials in general

5.1 General observations

The first step in the risk assessment of each substance is to check whether the existing risk assessment methodologies can also be used for the substance under consideration. Central points are the applicability of the methodology to characterize substance identity in a way that exposure of man and environment can be understood, to obtain relevant information to run exposure models, and to perform a meaningful risk characterisation by comparing the exposure estimates with the relevant toxicity endpoints. The case study on nanosilver first of all showed the need for a clear definition of a nanomaterial in REACH, discussions on which are still ongoing. The case study further showed several knowledge gaps in all aspects of risk assessment. Given that nanomaterials in general will exist in different sizes and shapes, in contrast to the case of nanosilver which was assumed to exist only in one particular size and shape, more problems may be expected, necessitating an even closer consideration of the applicability of basic risk assessment assumptions.

Case study disclaimer

In the presented nanosilver case study assumptions were made with regards to the size, shape, use and related exposure and tonnage level of nanosilver. Information on the hazardous effects from nanosilver and bulk silver were derived from a recent review by Wijnhoven et al. (2009) and information from CTGB (2004). It can not be excluded that if further literature searches and/or more information would have been available to the authors different conclusions might have been drawn. It is also stressed that if different assumptions were made regarding the form and shape of actual exposure to the various populations (consumer, man indirect) this might have led to different/additional questions. The present case study was focused at (some of) the issues one might encounter when a nanomaterial with specific use and size and its bulk form are to be registered under REACH.

5.2 Substance identification

5.2.1 Physicochemical properties

For a proper identification of any substance to be registered, including substances at the nanoscale, it is necessary to provide information on its physicochemical properties. For nanomaterials, special attention should in that respect be paid to granulometry: it is not only important to have data on particle size and size distribution, but also on *e.g.* shape, aspect ratio, specific surface area, and agglomeration/aggregation state. Also the solubility or dissolution characteristics of a nanomaterial need special attention, in dependence of its particle properties and the properties of the different media. It should be realised that nanomaterials interact with their environment, including interactions with environmental substrata (organic matter) and tissue culture media components, and that therefore temporal changes may occur (*e.g.* in size and size distribution). The characteristics as suggested by the OECD-WPMN (see also Appendix 2 under ‘Nanomaterial information/identification’ and ‘Physical-

chemical properties and material characterization') are briefly summarized in Table 5, where they are compared to the REACH information requirements in order to see if additional nano-specific requirements may be necessary.

Table 5. Comparison of the physicochemical characteristics of nanomaterials as suggested by the OECD-WPMN with the REACH information requirements, and proposal for additional nano-specific information requirements.

No.	Physicochemical characteristic	Included in REACH information requirements?	Comment	Additional nano-specific information requirement
1	Agglomeration/ aggregation	No	Agglomeration is only partly a physico-chemical characteristic; it significantly depends on the medium.	Yes
2	Catalytic properties	No	Not well defined.	No
3	Composition	Yes, Annex VI, section 2.3		No
4	Concentration	Yes, Annex VI, sections 2.3 and 3.4	This is not a physicochemical characteristic.	No
5	Crystalline phase	Yes, Annex VII, section 7.1		No
6	Dustiness	No		Yes
7	Fat solubility/ oleophilicity	Partly, Annex VII, section 7.8	Study does not need to be performed for inorganics, where instead an estimation method can be used that should be described.	Yes
8	Grain size	Yes, Annex VII, section 7.14		No
9	Hydrodynamic size/ particle size mea-surement/distribution	No		Yes
10	Length	Partly, related to Annex VI, sections 2.2.1 and 3.4	Length can be calculated from structural formula	Yes
11	Purity	Yes, Annex VI, section 2.3		No
12	Shape	Partly, related to Annex VI, sections 2.2.1 and 3.4	Shape can be calculated from structural formula.	Yes
13	Specific surface area	No		Yes
14	Surface charge/zeta potential	No	The zeta potential is dependent on both the medium where the substance is in and the properties of the substance itself. It then provides a measure of the ability to form aggregates. Zeta potential can be used as indicator for surface charge.	Yes
15	Surface chemistry	No	Not well-defined.	Yes
16	Water solubility/ hydrophilicity	Yes, Annex VII, section 7.7		No*

* Additional information may be required, since dissolution kinetics may be different for nanomaterials.

The physicochemical characteristics mentioned in Table 5 have been grouped, and for each group additional nano-specific requirements have been identified that are considered vital for a proper risk assessment (*e.g.* as input for fate modelling).

Group 1. Physicochemical characteristics relating to molecular dimensions

Composition (no. 3), crystalline phase (no. 5), dustiness (no. 6), grain size (no. 8), hydrodynamic size/ particle size measurement/distribution (no. 9), length (no. 10), purity (no. 11), shape (no. 12), and specific surface area (no. 13) can all be related to the molecular dimensions of a nanomaterial. Information on some of them is already required under REACH, leaving additional nano-specific information requirements for nos. 6, 9, 10, 12 and 13.

Group 2. Physicochemical characteristics relating to reactivity

Catalytic properties (no. 2), surface charge/zeta potential (no. 14), and surface chemistry (no. 15) can all be related to the reactivity of a nanomaterial. Since no. 2 is not well defined and appears to imply studying too many catalytic reactions, it is proposed to focus on nos. 14 and 15 as additional nano-specific information requirements.

Group 3. Physicochemical characteristics relating to agglomeration and aggregation

Agglomeration and aggregation (no. 1) as mentioned under the comment in the table are highly dependent on the medium, and not only depend on the substance properties. Examples are the stabilization of nanoparticles by dissolved organic carbon in water and the pH-dependence of agglomeration and aggregation. The information that one really needs to know is the colloidal properties of a solution of nanoparticles. Agglomeration and aggregation thus highly relate to grain size (no. 8), shape (no. 12), specific surface area (no. 13), surface charge/zeta potential (no. 14), and surface chemistry (no. 15). Information on some of them is already required under REACH, leaving additional nano-specific information requirements for nos. 12, 13, 14 and 15.

Group 4. Physicochemical characteristics relating to environmental fate and distribution

Fat solubility/oleophilicity (no. 7) and water solubility/hydrophilicity (no. 16) can be related to the environmental fate and distribution of a nanomaterial. Since information on no. 16 is already required under REACH, an additional nano-specific information requirement is proposed for no. 7.

What became clear from the case study is that, when a substance not only exists in the nanoform but also in bulk form, also the bulk form needs to be properly characterized. In the past, these bulk or non-nanomaterials were usually not well characterized as to their particle size/particle size distribution (nor was silver in the case study, for that matter). However, when a non-nanomaterial can have any dimension above 100 nm, it is very well possible that the behaviour of the smaller sized particles (*e.g.* 100 – 1000 nm range) is different from that of the larger sized particles (*e.g.* µm–mm range). So, both the nanoform and the bulk form need to be properly defined and characterized. There will always be differences between the two forms (so no ‘sameness’ in that sense, see also section 5.2.3), but these differences only matter when they impact behaviour and thus exposure and hazard. This information is essential to be able to conclude on whether or not read-across between the two forms is possible and justified.

In that respect, the form in which nanomaterials should be tested is also an important subject: is it in the form they will be available in the consumer product, human body, the environment et cetera (*e.g.* agglomerates) or in the form they are produced (as purified, stabilized, and free nanoparticles). A problem identified in the nanosilver case study related to that is that current analytical

methods/techniques may not be suitable for nanomaterials with variable surface chemistry, solubility and reactivity.

5.2.2 Impurities

Physical, chemical and toxicological properties of nanomaterials may depend on particle size. Since nanomaterials are normally produced in a narrow particle size range as this (among other aspects) determines their specific properties, it is important to know how to deal under REACH with nanoparticles that are outside the intended range, because they may contribute to the toxic effects. In the nanosilver case, the nanosilver was assumed to be produced in a narrow particle size range, so a rather monodisperse particle size distribution of 15 ± 5 nm was assumed. But when the distribution is much wider (*i.e.* multidisperse rather than monodisperse), the particles at both the lower and higher size range may contribute to the toxic effects, be it in a similar or different way than nanoparticles within the intended range.

As given in section 2.2, the definition of a substance under REACH goes beyond a pure chemical compound defined by a single molecular structure: it includes different constituents like impurities. Although the guidance on substance identification identifies the possible need for additional information on the size of substances in nanoform in order to properly characterize them, no guidance is yet given on how to address different sizes of a nanomaterial in substance identification. So, it is unclear at the moment how to deal with nanoparticles outside the intended range: are they to be considered as impurities or, because they consist of the same element, as part of the substance, making the substance possibly a multi-constituent substance? And how to deal with the mass-based cut-offs for specification of impurities (normally ≥ 1 % w/w, but impurities that are relevant for the classification and/or for PBT assessment always) and for mono- and multiconstituent substances (≥ 80 % w/w and ≥ 10 % – < 80 % w/w, respectively). These mass-based percentages may not be the most relevant measure for nanomaterials, as a small mass of nanomaterial still represents a large number of particles. Cut-offs based on *e.g.* volume, particle number or surface area may be more applicable.

It is to be noted that the definition of a substance under REACH, once the proper dose metrics has been established (see section 5.3), may need to be amended for nanomaterials to also include size and shape or other relevant characteristics.

5.2.3 Sameness discussion[26]

As noted in the introduction, for the majority of the presently known nanomaterials there are already bulk forms of these substances on the market. Most of these bulk forms will have to be registered under REACH before the 1st of December 2010. For these phase-in substances the issue of the sameness of the nanoform and bulk form is of extreme importance in view of the 'one substance – one registration' principle under REACH, and thus whether one or two registrations need to be submitted. Yet, as already indicated in chapter 1, this 'sameness' analysis is still a subject of discussion: there are no clear determinants for sameness yet, nor consensus on the minimum parameters/descriptors needed. Also the suggested use of information on physicochemical and (eco)toxicological properties in deciding on the sameness of a substance is problematic: information on many of these properties will neither be available nor required for a nanoform, and thus cannot be used in the assessment of (dis)similarity of properties between the bulk form and the nanoform. When in addition it is not clear yet what is the

[26] The statements in this paragraph are based on the status of the ongoing discussion on sameness as described in documents of the Competent Authorities Subgroup on Nanomaterials dated December 2008-March 2009.

amount and type of information that is considered essential to demonstrate (dis)similarity of properties, it appears that the 'sameness' analysis cannot be tackled properly at this point in time. It is to be noted that this problem not only holds for the nanoform vs. bulk form comparison, but also for the comparison between different sizes/shapes of a nanomaterial.

Moreover, another problem identified in the nanosilver case study is that even in such a data-rich dossier as for silver, it is difficult to decide on the sameness of nanosilver and (metallic) silver because the available *in vitro* and *in vivo* studies for both compounds differ for the most part in investigated parameters and exposure conditions. No doubt, this lack of comparative or 'bridging' studies will also form an obstacle in the sameness discussion for other nanomaterials than nanosilver. In order to make reliable comparisons between nanoform and bulk form possible in the future, comparative studies examining similar endpoints/parameters under similar exposure conditions (route, duration, way of application, et cetera) are highly recommended (see also section 5.5.3).

5.3 Dosimetry: mass-based or based on another metric?

For nanomaterials the actual metric that best describes the observed effects in test organisms or environmental fate and distribution may not be mass-based, *e.g.* expressed as mg/kg body weight or mg/L. There are indications that for example the number of molecules or nanoparticles, the surface area or another metric is a better one to relate to the observed fate, behaviour and effects. Using another metric, however, will need further discussion, and would also have lots of consequences, among others for other legislations. This includes the legislation on classification and labelling, where most hazards of a substance are related to mass concentration.

The point that mass may not the best scientifically defensible metric is not unique for nanomaterials. For example, for classification and labelling it is already practice that for some metals the massive form is classified and labelled differently than the metal in particle form, dependent on the particle or powder size. The underlying scientific reason is that only the dissolved metal is responsible for possible toxic effects. The rate at which metals dissolve and form the possible toxic ion species is very much dependent on the surface area to volume ratio. It is therefore that the OECD transformation/dissolution protocol (OECD, 2001) was developed to determine the rate and extent to which metals or sparingly soluble metal compounds can produce toxic bioavailable forms and whether this rate and extent of formation is of concern and should lead to classification. Whereas science describes that classification and labelling could thus be based on another metric, *i.e.* the combination of a surface area to volume ratio and the loading rate, the pragmatic way of dealing with this is to form classification bands, where massive metal above a certain diameter will be classified and labelled in one way, and metals with a diameter smaller than that are to be classified and labelled differently.

Making use of the best science and a pragmatic way of banding was thus a solution to deal with dosimetry issues for the classification and labelling of metals in different forms. Other solutions may be sought for nanomaterials where perhaps other metrics may be better from a scientific point of view, but where a 'translation' could take place to make it suitable for current systems that are based on mass metrics.

5.4 Exposure assessment

5.4.1 Exposure based waiving

Under certain conditions, exposure information can be used as a waiver under REACH for providing (eco)toxicological information. One of these conditions is that exposure is low/limited/not relevant/negligible. But the decision as to whether this is the case for a nanomaterial is difficult to make: not only is the definition of what is considered to be low/limited/not relevant/negligible exposure already a point of discussion for bulk forms of substances, it becomes even more difficult for nanomaterials because for nanomaterials the relevant dose metrics are unclear (see next paragraph). Furthermore, it is unclear whether the definition and the cut-off point should be different for a nanomaterial than for a bulk material. A possible concept for human exposure could be the Threshold of Toxicological Concern (TTC) concept: a principle which refers to the possibility of establishing a human exposure threshold value, below which there is no appreciable risk to human health by considering extensive databases of toxicity data generated in the past. It is to be noted though, that the TTC concept is already problematic for bulk substances, given that databases so far cover primarily systemic effects from oral exposure. Other exposure routes (like dermal and inhalation) are therefore not covered, and the potential use of TTCs for these routes needs to be further explored. That is even more the case for nanomaterials, for which databases to derive TTCs are completely lacking at the moment, for whatever exposure route.

5.4.2 Dosimetry

Another important issue is the expression of the metric of the exposure estimate. As already remarked in chapter 3 and section 5.3, number concentration and surface area are likely to be more appropriate for nanomaterials than mass concentration. Moreover, it should correspond to the metric of the nanomaterial dose used in the toxicological studies, in order to be able to compare them in risk characterisation.

5.4.3 Exposure modelling

In order to be able to do an exposure assessment for nanosilver, several assumptions on size, form, exposure route and exposed population had to be made. For a proper exposure assessment of nanomaterials, however, not only information on the frequency, duration and height of the exposure for all relevant routes of exposure and all human populations and environmental compartments exposed is important. It is also essential to know the size and form of the nanomaterial at the actual exposure site (which may depend on the mode of action). Nanoparticles may agglomerate, aggregate or change form during or after formulation into a consumer product. As a consequence, the product may not contain nanoparticles at all, or even nanoparticles with different characteristics than originally added in the manufacturing or formulation process. Also the conditions during exposure may affect the characteristics of the nanoparticle, for example the conditions in the gastrointestinal tract, in the air or on the skin. It is known that nanomaterials of different size, shape, et cetera, may display different kinetics and toxicity. The problem is, however, that at present little knowledge is available on processes like aggregation and agglomeration, and hence, these processes cannot be modelled (yet). Thus, for the time being, experimental research is required to obtain information about the form of the nanomaterials in the consumer product and during exposure.

For exposure assessment, models like EUSES, ConsExpo and EASE are currently in use for non-nanomaterials to provide exposure estimates for the environment, consumers and workers, respectively.

When trying to apply EUSES and ConsExpo in the nanosilver case, several issues were identified as to their suitability for nanomaterials.

Input for environmental exposure models (*e.g.* EUSES) are often based on QSPR calculations using physicochemical properties of the substance, mainly K_{OW} and K_p values. At the moment it is highly unlikely that these QSPRs will be applicable for nanomaterials. It is therefore recommended to not use QSPRs (including read-across approaches) to estimate properties of nanomaterials, as long as there is no (solid) basis to do so. Instead, measured partitioning coefficients (*i.e.* K_p values) can be used to estimate environmental distribution. When sufficient information on the fate and behaviour of nanomaterials becomes available it may either be concluded that the current QSPR estimations are applicable for nanomaterials as well, or new QSPRs for nanomaterials can be developed. At present, however, there is a need for specific information on nanomaterials (as indicated in section 5.2.1), especially on properties that are necessary for (estimating) fate and behaviour (*e.g.* K_p values) and (modelling) hazard characterisation. In addition, information that enables a proper comparison between bulk form and nanoform is lacking.

Also the exposure estimates generated for nanomaterials with ConsExpo should be interpreted with caution (and with the first tier model ECETOC TRA even more), as indicated by the nanosilver case study. This consumer exposure model was originally developed for human exposure assessment to normal, non-nanosubstances. Consequently, the different models within ConsExpo, and their corresponding assumptions, have not (yet) been evaluated for their applicability to nanomaterials. For example, nanoparticles may stay longer airborne. Also, the cut-off size for inhalation will likely be totally different for nanoparticles. For non-nanosubstances this cut-off size is 15 μm in ConsExpo, *i.e.* particles above that size are considered to be generally deposited in the upper airway regions, where most of them will be cleared to the gastrointestinal tract and where they will follow oral uptake kinetics. In contrast, particles smaller than 15 μm are considered to be generally deposited deeper down in the airways, like for instance in the pulmonary region, and there they will be absorbed locally. Whether that is also the case for nanomaterials needs to verified, because especially for nanoparticles the size-dependent deposition and absorption is an important issue. Also assumptions need to be developed for the fraction of nanoparticles that will pass the barrier of the skin (after dermal exposure) or the gastrointestinal tract (after oral exposure). It might even be necessary to create an additional route/barrier in ConsExpo, namely the olfactory route, because neuronal absorption was demonstrated for nanosilver via the olfactory nerve, and this may also be possible for other nanomaterials. As to dermal absorption, in ConsExpo this can be estimated with a QSAR[27]. This QSAR is already limitedly reliable for bulk substances, and hence should be evaluated thoroughly before use for nanoparticles. Until now, the assessment of the validity of an experimentally determined fraction is not part of the ConsExpo model, but still is an important aspect to consider when using a fraction absorbed in an exposure assessment.

Given the specific properties of nanomaterials it can thus be concluded that the existing exposure models need to be adapted in order to be ready for use for nanomaterials. At the moment, however, there is a lack of reference data to incorporate relevant nano-specific parameters into these models. The uncertainties about the specific form to which humans or the environment are exposed are largely unknown, which is further complicated by the fact that during their life-cycle nanomaterials can transform from one form to another (including the transformation from nanoform to bulk form). In addition they can form larger agglomerates, which may have different properties as well. For adaptation of the models, therefore, the following information appears to be essential:

[27] Quantitative Structure Activity Relationship.

- Information on the life-cycle of the specific nanomaterial. Especially information on the (nano)form to which humans or the environment are exposed is essential (*e.g.* as manufactured or as used).
- Information on the distribution of the (different) nanoform(s) over the environmental compartments (*i.e.* partition coefficients for sediment/water, soil/water and air/water may have to be measured).
- Information on the cut-off size for inhalation, deposition and absorption of nanoparticles.
- Information on the fraction of the particles that will pass the barrier of the skin and gastrointestinal tract for a proper estimation of the internal exposure.

Despite the gaps identified in the existing exposure models in parameters relevant for nanomaterials and the need for further development of these (and other) models, they can be used for the time being, as long as one considers the outcomes to be rough estimates that have to be interpreted with caution. The information generated can also be used to get a feeling for the relevance of the doses tested in toxicological studies. In the specific case of nanosilver, the rough estimates provided by ConsExpo were also useful for a 'quick and dirty' risk assessment (see section 4.3.4.3).

For environmental exposure, however, the nanosilver case study showed that suspended nanoparticles themselves could contribute to toxicity in addition to the dissolved (Ag^+) fraction. This indicates that the absence of a proper estimation of environmental distribution (*e.g.* based on measured K_p values) strongly hampers the estimation of a (worst-case) environmental exposure scenario.

5.5 Hazard assessment

5.5.1 Dosimetry

REACH uses several triggers for classification and labelling, to identify PBT/vPvB substances and for further testing (*e.g.* secondary poisoning). These triggers are often based on mass concentration. This may not be useful for nanomaterials because a small mass still represents a high number of particles. However, until adapted to the relevant dose metric for nanomaterials, mass concentration is also to be used for nanomaterials for these triggers.

The issue of dosimetry is in any case very important for nanomaterials, also in (eco)toxicity testing. As already remarked in chapter 3 and section 5.3, number concentration and surface area are likely to be more appropriate for nanomaterials than mass concentration. Besides, with the small particle sizes of nanomaterials and the normal dosimetric in toxicity studies (mass concentration), there is a distinct possibility that due to the large amount of nanomaterial to be administered (which is no longer representative for the actual exposure situation) toxic effects induced are a consequence of an overload phenomenon or physical stress, rather than a toxic effect following exposure to the nanomaterial itself.

The dosimetry not being clear for nanomaterials also affects the derivation of NOAELs and DNELs for human health and of NOECs and PNECs for the environment. REACH requires these measures of dose-response to be established for use in risk characterisation, but how to express these measures and how that relates to the dose metric normally used for the bulk form is uncertain.

5.5.2 Derivation of DNELs and PNECs

Aside from the dosimetry issue, an additional issue identified is the uncertainty whether the procedure for deriving DNELs and PNECs as established for non-nanomaterials is also applicable for nanomaterials. Basically, these procedures come down to converting measures of dose-response in

toxicity studies (like for instance NOAELs and NOECs) into DNELs and PNECs by application of assessment factors. These assessment factors address uncertainties in the extrapolation of experimental data to the real exposure situation, taking into account variability and uncertainty. For non-nanomaterials, standard default assessment factors for inter- and intraspecies differences, for differences in exposure duration, for dose-response issues and for quality of the database have been established, based on common regulatory practice and comprehensive toxicological database analyses. Since neither of these is available for nanomaterials at the moment, it is not possible to judge whether it would be justified to apply the standard default assessment factors for non-nanomaterials also to nanomaterials.

5.5.3 Non-testing methods

Under REACH, the use of non-testing methods like (Q)SARs and grouping/read-across is propagated. The question is, however, how far these can be applied to nanomaterials, since already for bulk substances for most toxic endpoints *e.g.* (Q)SARs are not well validated at the moment due to lack of proper reference databases. For nanomaterials that will be even more the case. In addition, the nanosilver case study has pointed out that also the application of read-across is not so simple, given the 'sameness' discussion (see section 5.2.3). Even for such a data-rich compound as silver, it was not possible to decide on the sameness of nanosilver and (metallic) silver because the available *in vitro* and *in vivo* studies for both compounds differed for the most part in investigated parameters and exposure conditions. Hence, without comparative or 'bridging' studies, in which for the nanomaterial and the corresponding bulk material comparable parameters are investigated under comparable conditions (preferably in the same study), it will be very difficult to come to a definitive conclusion on the 'sameness' of nanoform and bulk form. Given that most presently known nanomaterials are derivatives of existing bulk materials, the option of read-across (*i.e.* the use of data in the registration dossier for the bulk form also for registration of the nanoform) will thus not be a viable option when 'bridging' studies are lacking. Comparative studies examining similar endpoints/parameters under similar exposure conditions (route, duration, way of application et cetera) are therefore highly recommended (see also section 5.2.3). This recommendation applies both to *in vitro* and *in vivo* studies.

5.5.4 *In vitro* methods

Given the rate of development of new nanomaterials and the simultaneous pressure to gain knowledge on the potential hazards of nanomaterials, the current view is that traditional risk assessment procedures, with an important role for *in vivo* studies, would unlikely be able to keep up. *In vitro* studies have therefore been put forward as a faster approach to screen nanomaterials for their toxic potential. This suggested use of *in vitro* screening studies for nanomaterials would fit well with the propagated use of *in vitro* methods under REACH, also in terms of costs and animal welfare. From a scientific point of view though, it is not so evident that at this point in time *in vitro* studies can be granted such an important role in the risk assessment of nanomaterials: a comparative analysis of published *in vitro* data with nanomaterials has shown that there are several knowledge gaps and issues that first need to be resolved (Park et al., 2009). A number of these gaps and issues are associated with the *in vitro* assay protocol choices and with dose response analysis. With respect to the former, little is known on the effect of exposure and dispersion methods on the outcome of *in vitro* toxicity assays. Also, currently available *in vitro* methods only address a limited number of toxic endpoints (cytotoxicity, oxidative stress, inflammation and genotoxicity), and the choice of readout system, cell type and exposure duration have been shown to affect their outcome. Especially the choice of cell type appears in general, for all toxicity endpoints, to be a major contributing factor, given the differences in uptake and removal of nanomaterials by cells, and the possible involvement of specific cell functions. With respect to dose response analysis, the identification of the appropriate dosimetry for

nanomaterials is important. It has been questioned whether the general use of nominal concentrations for representing results in *in vitro* studies is proportionate to the actual dose delivered to the cells to the same extent for every nanomaterial (Park et al., 2009). Problem of a more general nature is of course the extrapolation of *in vitro* toxicity data to the *in vivo* situation. For nanomaterials this is even more problematic than for non-nanomaterials, because at this stage no proper reference database for *in vivo* effects of nanomaterials is available, making it difficult to assess the robustness and relevance of several *in vitro* methods. In order to get information on the relevance and robustness of *in vitro* methods, that are considered essential for the development of proper screening methods for future use, it is highly recommended to run well defined *in vivo* studies on the bulk and the nanoforms in parallel with *in vitro* studies. In the present case study, *e.g.* well defined and comparable kinetic and repeated dose inhalation studies (preferably including kinetics) would have been of help and could have been a good starting point for the sameness assessment for the human health endpoints.

5.5.5 Data requirements

Due to the specific properties of nanomaterials, it may be expected that their behaviour, distribution, and effects differ from the bulk substance. Therefore, other toxicity endpoints may be of importance than normally studied in standard toxicity tests.

For human health hazard assessment it became clear from the nanosilver case that, although no standard requirement under REACH, it would for nanomaterials in general be desirable to have data on kinetics, first of all to determine whether or not the nanomaterial can be transported across the portals of entry. When the nanomaterials are not passing the barriers and are not becoming systemically available (requiring a definition of what is 'not available' and 'not considered of toxicological concern', in the proper dose metrics, see section 5.5.1), the focus for further investigations can only be on the potential local toxicity of the nanomaterial. But if the nanomaterial can become systemically available, then kinetic studies can be of help in determining in what entity (or entities) it becomes systemically available, is distributed in the body and exerts its effect(s), and how that is influenced by different exposure routes or by different sizes and shapes of the nanomaterial. Kinetic information can also be of great use in the 'sameness' analysis (*i.e.* on the dissimilarity/similarity of the toxic entity/entities between the bulk and the nanomaterial) and to direct further (toxicological) investigations at the right target organs at higher tiers. Most likely there will be a 'turning point' in the nano-characteristic behaviour of the nanomaterial: above this point it will behave like the bulk material, below this point the nano-characteristics will come into play. In that case, the nanomaterial is to be seen as a new chemical entity, for which additional research is necessary, *e.g.* into interactions on cellular level and into passage over specific barriers or via specific transport (blood–brain, blood–testes, placenta, mother's milk, neuronal transport). Question is, however, whether this 'turning point' can actually be determined, given also the problems identified in the nanosilver case with the suitability of current analytical methods/techniques.

Also for environmental hazard assessment kinetic information is of importance, especially in relation to fate related properties, such as water solubility, (dis)aggregation/(dis)agglomeration and (bio)transformation. As shown in the nanosilver case study toxicity can be the result of suspended nanosilver and dissolved silver ions. Similarly for other nanomaterials the rate and extent of dissolution or disaggregation/disagglomeration can influence availability and toxicity (and to what extent this differs between bulk form and nanoform). Furthermore, persistence of the nanomaterial and (bio)transformation products may influence the toxic potential. To tackle questions on (bio)availability and (bio)transformation a sediment/water test (*e.g.* OECD Test Guideline 308) may be useful. Within REACH (and other risk assessment frameworks), however, the need for (hazard) information depends on the type of nanomaterial and the extent in which it is released into the environment (if a

nanomaterial is only used in a closed system in a factory, fate and behaviour in the environment are not of importance).

For nanomaterials without corresponding bulk form, concern has been expressed that due to the low tonnage level in which they are manufactured or imported, either they are excluded from registration (when below the 1 tonne threshold for registration) or registration will only provide a limited amount of information because of the low tonnage data requirements. When looking at the human health hazard data requirements (see Appendix 1), the key information that is lacking at the 1 – 10 tonnage level is repeated dose toxicity testing, compared to higher tonnage levels. That, however, is not different for a nanomaterial and a bulk material. What possibly differs is that for nanomaterials the proper dose metric may not be mass concentration. This may make it necessary to adapt the tonnage bands for nanomaterials once the proper dose metric has been established.

Under REACH only a limited set of aquatic tests is required for lower tonnage levels (1 – 10 tonnes/year): growth reduction in algae, and short-term *Daphnia* and fish tests, with the addition that for substances with a low water solubility (*i.e.* most nanomaterials) chronic (*Daphnia*) testing should be considered. In addition, at present a lack of data on (toxicity) of nanomaterials triggers the need for additional tests with fish.

To establish PBT/vPvB properties of a substance, a bioaccumulation study is necessary, because for now a BCF value for nanomaterials cannot be estimated using a QSAR. Furthermore, there is a need for long-term (fish) toxicity data, because at lower tonnage levels (< 100 tonnes/year) this information is not required and the commonly used extrapolation is (possibly) hampered by a lack of knowledge on the specific mode of action of nanomaterials. Similarly, additional information may be needed on toxicity of nanomaterials in soil, at least for the evaluation of extrapolation models.

5.6 Risk characterisation

In risk characterisation, exposure levels for environmental compartments and human populations exposed are to be compared to environmental and human health hazard information, *i.e.* PNECs and DNELs, respectively. The focus of the risk assessment under REACH, however, is on risk management, *i.e.* on the demonstration that risks are controlled ('safe use') for both the human target populations (workers, consumers and humans exposed via the environment) and the environmental compartments (aquatic, terrestrial and atmospheric) to be protected. Risks are regarded controlled when the estimated exposure levels do not exceed the DNELs or PNECs (*i.e.* ratio exposure/DNEL or exposure/PNEC should be < 1). Consequently, when these ratios are above 1, iterations should follow on either the hazard side or the exposure side or a combination thereof, until the ratios become < 1. Iterations could for example include refining the hazard assessment by obtaining more data, refining the exposure assessment by using monitoring data or more realistic modelling, or refining the conditions of manufacture or use by *e.g.* introducing more stringent risk management measures (RMMs) or changing the operational conditions (OCs).

For nanomaterials, three problems have been identified with this concept of risk characterisation under REACH. The first one is the relevant dose metric for the exposure estimates (see section 5.4.2) and the derivation of DNELs and PNECs (see section 5.5.1), the second on the appropriateness of the DNEL- and PNEC-derivation procedures (see section 5.5.2).

The third problem concerns iterations on the exposure side. The ultimate aim under REACH is to identify and document the conditions of manufacture and use which are needed to control the risks to

human health and the environment in an exposure scenario. An exposure scenario therefore includes OCs (*e.g.* duration and frequency of use, amount or concentration of a substance in an activity, process temperature) and RMMs (*e.g.* local exhaust ventilation, wearing certain types of gloves). As indicated above, one of the options for iteration on the exposure side is to introduce or improve OCs and/or RMMs, resulting in lower exposure levels. Such iterations must be realistic to the extent that these OCs and/or RMMs can be implemented in practice. Question is whether the demonstration of 'safe use' is possible for nanomaterials at this point in time, since strategies for controlling exposure to nanomaterials are still under development (especially in the workplace situation) and there is no idea yet of what would be realistically implementable and sufficient OCs and RMMs for nanomaterials for the workplace, the consumer or the environment.

5.7 Lessons learned with respect to existing risk assessment frameworks

For the specific case of nanosilver as described in chapter 4, relatively many data, both *in vitro* and *in vivo*, were available on the nanomaterial itself, as well as on the bulk material. From these data it could be concluded that nanosilver becomes systemically available and that it exerts effects. But despite silver being a data-rich compound, a conclusion on the 'sameness' of these effects between nanosilver and bulk silver appeared to be too difficult. So, in trying to identify the data gaps for nanosilver and what kind of studies could/should be done to fill these gaps, the currently proposed risk assessment approaches for nanomaterials were evaluated (see section 3.3) for inspiration. Unfortunately, the one proposed by SCENIHR (2007b; consisting of four stages) was not of much help for nanosilver: with the kind of data available for nanosilver the first two (exposure-oriented) stages were in fact already passed, which brings the assessment already deep in the third stage of hazard identification (see Appendix 4). More importantly, SCENIHR does not specify exactly what kind of *in vitro* and/or *in vivo* testing is to be performed in order to provide the necessary (screening) information for nanomaterials, nor accounts for the difficulties identified in *in vitro* testing for nanomaterials (see section 5.5.4).

The latter is also the case for the framework formulated by Howard and De Jong (2004) (see section 3.3). This framework is in fact more a prioritizing tool than a risk assessment tool, and is based on the combination of a couple of physicochemical characteristics of the particle and some specified hazard triggers, without specifying the kind of tests that could provide the screening information on these triggers. Moreover, when the specified hazard triggers are not known for a nanomaterial, which was in fact the case for nanosilver for most triggers, it is given a high priority for risk assessment. And since that may be the case for most nanomaterials, it is questionable whether the framework really can be used as a prioritizing tool. Besides, no matter how high or low the priority, the necessary studies for completing the risk assessment for a nanomaterial are not specified in the framework.

Another lesson learned is that in the abovementioned frameworks the question 'is there relevant exposure or is exposure considered to be low' forms an important initial step. In order for this question also to be applicable under REACH further attention should be paid to this issue to clarify its uncertainties. On the one hand, the decision as to whether exposure to nanomaterials is low/limited/not relevant/negligible (*e.g.* necessary for exposure based waiving) is difficult to make as long as the dose metric for nanomaterials is unclear and there are no reference databases (yet) that provide insight at which dose levels/metrics adverse effects occur/do not occur. On the other hand, as noted in section 2.5, under REACH a detailed exposure assessment is only required for substances in production/import volumes over 10 tonnes/year that are classified as dangerous or PBT/vPvB. As a consequence, relevant information on exposure will probably be lacking for most of the nanomaterials, which makes it difficult to apply the risk assessment frameworks developed and described so far.

5.8 Conclusions

Although the framework for risk assessment of chemicals as outlined in the legal text of REACH in principle should also be applicable to nanomaterials, the following issues have been identified that need further attention before all the concepts in REACH can be properly applied to nanomaterials in the future.

1. In REACH, a definition relating to nanomaterials has not been laid down. This, however, is considered essential for the scope of risk assessment of nanomaterials.
2. A proper characterisation of a nanomaterial is considered essential for the determination of the dose metrics, fate and behaviour and exposure characteristics of the nanomaterial. This requires more information on physicochemical properties (*e.g.* on size and size distribution, shape, specific surface area, agglomeration/aggregation state and dissolution characteristics) than now required under REACH. When there is also a bulk form of the nanomaterial, additionally this bulk form needs to be properly characterized for bridging purposes.
3. Further guidance is needed on how to address different sizes and size distributions of a nanomaterial in substance identification. For a proper identification of a nanomaterial clarity should be provided on how to deal with nanoparticles outside the intended range (as impurity, or as part of the substance?).
4. The relevant dose metric for nanomaterials needs to be established since mass concentration, the standard dose metric for non-nanomaterials, may not be appropriate for all endpoints. This is important for:
   - exposure assessment, both for exposure estimation as well as for defining what is low/limited/not relevant/negligible exposure;
   - (eco)toxicity testing, *in vitro* and *in vivo*;
   - the setting of NOAELs and NOECs and the derivation of DNELs and PNECs;
   - the various triggers to be used under REACH. One such important trigger is classification and labelling, based on which (among other things) a detailed exposure assessment is to be carried out or not, and which also forms a trigger for other processes than registration under REACH. The cut-off values for most toxicological endpoints in classification and labelling, just like for other triggers as *e.g.* PBT/vPvB properties and cut-offs for specification of impurities and for mono- and multiconstituent substances, are often based on mass concentration and this may need to be adapted for nanomaterials.

   As long as the relevant dose metric has not been established, mass concentration should be used for nanomaterials (with caution).
5. For a nanomaterial with a corresponding bulk form, it is difficult to decide on the sameness of the nanoform and the bulk form with the instruments currently available under REACH. As a consequence, the propagated use of non-testing methods like read-across (*i.e.* data in the registration dossier for the bulk form are used for registration of the nanoform as well) will not be a viable option. In order to enable reliable comparisons between nanoform and bulk form in the future, there is an urgent need for comparative studies, *i.e.* studies examining similar endpoints/parameters for the nanomaterial and the corresponding bulk material under similar exposure conditions (route, duration, way of application et cetera), preferably in the same study.
6. The propagated use of *in vitro* (screening) methods under REACH is at the moment not a viable option for nanomaterials, due to a number of knowledge gaps and issues associated with the *in vitro* assay protocol choices for nanomaterials and with dose response analysis that first need to be resolved. Aside from these ‘technical’ problems, there is the more general issue of the

extrapolation of *in vitro* toxicity data to the *in vivo* situation. This is even more problematic for nanomaterials than for non-nanomaterials, because at this point in time there are no proper reference databases for *in vivo* effects of nanomaterials. This makes it difficult to assess the robustness and relevance of current *in vitro* methods. In order to develop proper *in vitro* screening methods for nanomaterials in the near future, it is highly recommended to run well defined *in vivo* studies on the bulk and the nanoforms in parallel with *in vitro* studies.

7. Kinetic information is not a standard requirement under REACH but for nanomaterials it is considered essential to both human health and environmental hazard assessment. For the environment, it is of special importance in relation to fate-related properties and for providing information on (bio)transformation and dissolution potential. For human health, it can provide information on whether or not the nanomaterial can be transported across the portals of entry and, when it can become systemically available, in what entity it is distributed in the body and exerts its effect(s). Kinetic information is also very important for the 'sameness' analysis (not only between the nanomaterial and the corresponding bulk form, but also between different sizes/shapes of a nanomaterial) and for directing further (toxicological) investigations at the right target organs. When over the next few years the generated kinetic data would allow the establishment of certain patterns in the behaviour of nanomaterials and how that is influenced by size and shape, it may no longer be necessary to require kinetic information for all nanomaterials.
8. With respect to (eco)toxicity testing, it can be concluded that most currently existing OECD test guidelines can be used for investigating the effects of nanomaterials, with the important proviso that additional consideration is needed for:
   - the physicochemical characteristics of the material tested and the influence of that on sample preparation and dosimetry;
   - modification of some test guidelines as to extension of parameters to be tested. For human health, this applies in particular to studies using the inhalation route and to toxicokinetic studies. For environment, this applies in particular to studies where different environmental compartments are present in the test system (*e.g.* water/sediment tests).
9. Existing exposure models need to be adapted and developed further, in order to be ready for use for nanomaterials. For this, the generation of reference data is essential, so that relevant nano-specific parameters can be incorporated into these models.
10. In order to do a proper risk characterisation and to be able to demonstrate 'safe use' for nanomaterials the following is considered necessary:
    - examination of whether the standard default assessment factors to be applied in the DNEL- and PNEC-derivation procedures are appropriate for nanomaterials; and
    - development of strategies for controlling exposure to nanomaterials, and investigation into realistically implementable and sufficient OCs and RMMs for nanomaterials for the workplace, the consumer and the environment.

6 Proposal for a risk assessment framework for first generation nanomaterials under REACH

6.1 Observations and considerations

The approach proposed in section 6.2 is based on the lessons learned from the case study on the first generation nanomaterial nanosilver, and in particular on the observations that at this point in time:

- there is no definition relating to nanomaterials under REACH, whereas this is urgently needed;
- the 'sameness' analysis can, as yet, not be properly tackled under REACH, and thus the read-across from data on the bulk form of a substance to its nanoform will be very troublesome;
- a nanomaterial cannot be properly characterized with the data normally required under REACH, and it is unclear how to address different sizes of a nanomaterial in substance identification;
- the relevant dose metrics for nanomaterials is not clear yet, but the standard dose metric for non-nanomaterials, mass concentration, may not be the most appropriate one. If indeed in the future another metric is established for nanomaterials, this needs to be implemented in REACH, as well as in other related legislation;
- there is no definition of what is low/limited/not relevant/negligible exposure for nanomaterials, partly due to the fact that a relevant dose metric for nanomaterials has not been established yet;
- existing exposure models can be run for nanomaterials, but there is a large degree of uncertainty in the outcomes as the models have not (yet) been validated for nanomaterials;
- there are no readily available *in vitro* screening methods at hand of which the robustness and relevance for *in vivo* effects of nanomaterials has been assessed.

In this approach consideration was further given to the fact that:

- it is questionable whether the various mass-based triggers under REACH (among which those for classification and labelling), as well as the tonnage bands for data requirements, are appropriate for nanomaterials (a small mass/tonnage still represents a large number of nanoparticles);
- to date, exposure to nanomaterials has hardly been investigated, resulting in very little information and knowledge on exposure patterns and on what sizes and forms of a nanomaterial humans and the environment are actually exposed to;
- likewise, there is as yet no reference database for *in vivo* effects of nanomaterials relevant to human health and the environment.

6.2 Proposed approach for the risk assessment of first generation nanomaterials under REACH

On the basis of the observations and considerations described in section 6.1, for the risk assessment of first generation nanomaterials under REACH it is proposed

1. to apply the registration requirements according to Annexes VI and XI of REACH (just like for non-nanomaterials);

2. to keep for nanomaterials the tiered concept in information requirements on physicochemical and (eco)toxicological properties, *i.e.* further testing in case of positive results, but not the staggered approach in information requirements with tonnage bands (as is for non-nanomaterials, and represented in Annexes VII–X of REACH) since the scientific need to ask for nano-specific information is independent of tonnage, which may not even be an appropriate measure for nanomaterials anyway;
3. to reconsider for nanomaterials the 1 tonne per year threshold for registration;
4. to apply as a first tier for all nanomaterials to be registered and independent of their production/import volume, a base set information requirement that covers the primary information needs on nanomaterials (see Text box 1);
5. to replace the linkage between detailed exposure assessment and tonnage and classification as dangerous or PBT/vPvB (as is for non-nanomaterials, and represented in Annex I of REACH) by a base set information requirement for a detailed exposure assessment, for all nanomaterials to be registered, independent of their tonnage (see Text box 1);
6. to consider, on a case-by-case basis, the need for further more targeted information in a next tier (or tiers), depending on the results of the base set studies (see Text box 2);
7. to apply the same base set data requirements to a nanomaterial for which there is already a bulk form on the market as to a nanomaterial without a corresponding bulk form;
8. to use this approach at least for the next few years, until further investigations have provided more clarity on the issues identified above. In that respect, it is also considered necessary that for all nanomaterials to be registered a technical dossier is provided plus, independent of their tonnage, a CSR/CSA. The generated data hopefully will allow certain patterns in the behaviour of nanomaterials to be established, on the basis of which, in time, the data requirements for nanomaterials can be adapted.

It is realised that at the moment there is no definition relating to nanomaterials under REACH. Work on the terminology is currently ongoing at different levels (*e.g.* OECD). For the meantime it is proposed to use the SCENIHR definition of a nanomaterial (*i.e.* any form of a material that is composed of discrete functional parts, many of which have one or more dimensions of the order of 100 nm or less) as working definition, as also suggested by the REACH Competent Authorities and its Subgroup on Nanomaterials.

It is also realised that the issue on dosimetry is very crucial and critical for the risk assessment of nanomaterials. Since the OECD-WPMN is currently already developing a relevant dose metric for nanomaterials, for the meantime it is proposed to use mass concentration for nanomaterials (with caution). At the same time, though, it is proposed to require information on nano-specific characteristics, in order to be able to establish relationships between mass and other metrics which could possibly be used in a pragmatic way (banding) for the time being.

Finally, it is realised that the scientific exercise described in this report concerned one nanomaterial only. It is recommended that more case studies are undertaken, with different types of nanomaterials (including non-metallic ones), to generate even more input that could be of help for policy makers when developing guidance on how to deal with first generation nanomaterials under REACH or other legislation.

Text box 1. Proposed base set information requirements for nanomaterials.

PHYSICOCHEMICAL PROPERTIES

– Annex VII requirements, including one nano-specific addition to water solubility:
  - Dissolution kinetics

In addition, the following nano-specific requirements:

– Dustiness
– Fat solubility/oleophilicity
– Hydrodynamic size/particle size measurement/distribution
– Length
– Shape
– Specific surface area
– Surface charge/zeta potential
– Surface chemistry

TOXICOLOGICAL INFORMATION *)

– Toxicokinetic testing
– Repeated dose toxicity testing for the inhalation route (or other routes, depending on the anticipated exposure routes), preferably with inclusion of additional parameters to the standard repeated dose toxicity study, such as cardiovascular and/or inflammatory parameters. It is also recommended to include kinetic parameters in the repeated dose toxicity study.
– *In vitro* gene mutation study and *in vitro* cytogenicity study

ECOTOXICOLOGICAL INFORMATION *)

– Algal growth test
– Chronic *Daphnia* test
– Information on the fate and behaviour, with special attention to
  - Stability of the nanomaterial: to what extent is it susceptible for (bio)transformation and which (bio-) transformation products are formed (and at which rate)?
  - Partitioning coefficients (Kp values)

EXPOSURE INFORMATION *)

– Information aimed at developing exposure scenarios and generation of exposure estimates, in accordance with Annex I of REACH, with special attention to
  - the frequency, duration and height of the exposure for all relevant routes of exposure and all human populations and environmental compartments exposed
  - the life-cycle, especially on the (nano)form to which humans or the environment are exposed (e.g. as manufactured or as used)

Note: exposure models and related parameters may need to be adapted.

*) Starting point for all testing is the adequately characterized nanomaterial. During testing there should be continuous monitoring and measuring of any changes occurring in the nanomaterial characteristics.

Text box 2. Proposed higher tier information requirements for nanomaterials.

In a next tier, further more targeted information might be considered necessary as a result of the base set studies. Further (eco)toxicological information may include, on a case-by-case basis, *e.g.*

- *in vivo* mutagenicity testing
- reproductive toxicity testing
- a sensitisation study
- a fish bioaccumulation study
- a chronic fish test

Note: for a nanomaterial for which there is already a bulk form on the market, the base set is primarily aimed at assessment of sameness. If sameness can be concluded, no further information may be needed following this first tier testing when sufficient data are available on the bulk form from which read-across can be applied. If sameness cannot be concluded, or if there is no corresponding bulk form on the market, the normal REACH requirements (Annex VII–X) will generally apply following the base set testing.

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Appendix 1

Standard information requirements (X) under REACH for substances manufactured or imported in quantities of one tonne or more (Annex VII), 10 tonnes or more (Annex VIII), 100 tonnes or more (Annex IX) and 1000 tonnes or more (Annex X) (EU, 2006)

		ANNEX VII VOLUME ≥ 1 t/yr	ANNEX VIII VOLUME ≥ 10 t/yr	ANNEX IX VOLUME ≥ 100 t/yr	ANNEX X VOLUME ≥1000 t/yr
PHYSICOCHEMICAL PROPERTIES					
7.1	State of substance at 20 °C and 101.3 kPa	X			
7.2	Melting/freezing point	X			
7.3	Boiling point	X			
7.4	Relative density	X			
7.5	Vapour pressure	X			
7.6	Surface tension	X			
7.7	Water solubility	X			
7.8	Partition coefficient *n*-octanol/water	X			
7.9	Flash-point	X			
7.10	Flammability	X			
7.11	Explosive properties	X			
7.12	Self-ignition temperature	X			
7.13	Oxidising properties	X			
7.14	Granulometry	X			
7.15	Stability in organic solvents and identity of relevant degradation products			X	
7.16	Dissociation constant			X	
7.17	Viscosity			X	
TOXICOLOGICAL INFORMATION					
8.1	Skin irritation/corrosion – *in vitro*	X			
8.1.1	Skin irritation – *in vivo*		X		
8.2	Eye irritation – *in vitro*	X			
8.2.1	Eye irritation – *in vivo*		X		
8.3	Skin sensitisation	X			
8.4.1	*In vitro* gene mutation study in bacteria	X			
8.4.2	*In vitro* cytogenicity study in mammalian cells or *in vitro* micronucleus study	(X)	X		
8.4.3	*In vitro* gene mutation study in mammalian cells	(X)	X		
8.4	*In vivo* mutagenicity studies	(X)	(X)	(X)	(X)

		ANNEX VII VOLUME ≥ 1 t/yr	ANNEX VIII VOLUME ≥ 10 t/yr	ANNEX IX VOLUME ≥ 100 t/yr	ANNEX X VOLUME ≥1000 t/yr
8.5.1	Acute oral toxicity	X			
8.5.2	Acute inhalation toxicity		X (or dermal)		
8.5.3	Acute dermal toxicity		X (or inhalation)		
8.6.1	Short-term repeated dose toxicity study (28 days)		X	(X)	
8.6.2	Sub-chronic toxicity study (90 days)		(X)	X	
8.6.3	Long term toxicity study (≥ 12 months)				(X)
	Further studies		(X)	(X)	(X)
8.7.1	Screening for reproductive/developmental toxicity (OECD 421 or 422)		X		
8.7.2	Pre-natal developmental toxicity study		(X)	X	X
8.7.3	Two-generation reproductive toxicity study		(X)	(X)	X
8.8.1	Assessment of the toxicokinetic behaviour of the substance to the extent that can be derived from the relevant available information		X		
8.9.1	Carcinogenicity				(X)
ECOTOXICOLOGICAL INFORMATION					
9.1.1	Short-term toxicity testing on invertebrates (preferred species *Daphnia*)	X			
9.1.2	Growth inhibition study aquatic plants (algae preferred)	X			
9.1.3	Short-term toxicity testing on fish		X		
9.1.4	Activated sludge respiration inhibition testing		X		
9.1.5	Long term toxicity testing on invertebrates (preferred species *Daphnia*)			X	
9.1.6	Long term toxicity testing on fish			X	
9.2.1.1	Biotic degradation – Ready biodegradability	X			
9.2.1.2	Simulation testing on ultimate degradation in surface water			X	
9.2.1.3	Soil simulation testing			X	
9.2.1.4	Sediment simulation testing			X	
	Further biotic degradation testing				(X)
9.2.2.1	Abiotic degradation – Hydrolysis as function of pH		X		
9.2.3	Identification of degradation products			X	
9.3.1	Adsorption/desorption screening study		X		
9.3.2	Bioaccumulation in aquatic species, preferably fish			X	
9.3.3	Further information on absorption/desorption			(X)	

	ANNEX VII VOLUME ≥ 1 t/yr	ANNEX VIII VOLUME ≥ 10 t/yr	ANNEX IX VOLUME ≥ 100 t/yr	ANNEX X VOLUME ≥1000 t/yr
9.3.4 Further information on the environmental fate and behaviour and/or degradation products				(X)
9.4.1 Short-term toxicity to invertebrates			X	
9.4.2 Effects on soil micro-organisms			X	
9.4.3 Short-term toxicity to plants			X	
9.4.4 Long-term toxicity testing on invertebrates				X
9.4.6 Long-term toxicity testing on plants				X
9.5.1 Long-term toxicity to sediment organisms				X
9.6.1 Long-term or reproductive toxicity to birds				X
METHODS OF DETECTION AND ANALYSIS				
Description of analytical methods for the relevant compartments for which studies were performed			X	X
OTHER AVAILABLE AND RELEVANT PHYSICOCHEMICAL, TOXICOLOGICAL AND ECOTOXICOLOGICAL INFORMATION	X			

Appendix 2

List of 'endpoints for testing nanomaterials' according to OECD-WPMN (OECD, 2008b)

Nanomaterial Information/Identification

Nanomaterial name
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 Characterization

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)

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
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

Appendix 3

Suitability of OECD test guidelines for the testing of nanomaterials (copied from Rocks et al., 2008)

		Suitable for use with nanomaterials	Concerns over use with nanomaterials	Unsuitable for use with nanomaterials
A.	**Physicochemical properties**			
	Substance state			
	Melting freezing temperature (OECD TG102)			Capillary method in liquid bath or in metal block (visual identification)[1]
				Kofler hot bar (visual identification)[1]
		Melt microscope (using microscope hot stages)		
		Method to determine the freezing temperature (temperature measured)		
		Apparatus with photocell detection		
		Differential Thermal Analysis (DTA)		
		Differential Scanning Calorimetry (DSC)		
	Boiling point (OECD TG103)	Ebulliometer		
		Dynamic method		
		Distillation method		
		Method according to Siwoloboff		
		Photocell detection		
		Differential Thermal Analysis (DTA)		
		Differential Scanning Calorimetry (DSC)		
	Relative Density* (OECD TG109)	Hydrostatic balance[2]		
	** nanomaterials assumed to be solid and not liquid*	Pycnometer[2]		

		Suitable for use with nanomaterials	Concerns over use with nanomaterials	Unsuitable for use with nanomaterials
	Relative Density (continued)	Air comparison pycnometer[2]		
	Vapour Pressure (OECD TG104)		Dynamic method (Cottrell's method, only if low melting point)	
		Static method		
		Isoteniscope		
			Effusion method: vapour pressure balance[2]	
			Effusion method: loss of weight[2]	
		Gas saturation method		
		Spinning rotor		
	Surface tension (OECD TG115)		Plate method[4]	
			Stirrup method[4]	
			Ring method[4]	
	Water Solubility (OECD TG105)[5]			Preliminary test (visual determination of dissolved amount)
		Column elution method		
			Flask method	
	Partition Coefficient (*n*-octanol/water)		Shake-flask method (OECD 107)[3,5,8]	
			HPLC method[3,5,8]	
	Flash point			Liquids only
	Flammability (solids)		Preliminary screening test[2]	
			Burning rate test (NF T20-042)[2]	
	Flammability (contact with water)	Step-by-step testing (not suitable for substances that spontaneously combust with air)		

		Suitable for use with nanomaterials	Concerns over use with nanomaterials	Unsuitable for use with nanomaterials
	Self-ignition Temperature (Pyrophoric)		Powdery solid poured from height and observed (NF T20-039)[2,6]	
		Relative Self-ignition Temperature (solids; NF T-20-036)		
	Explosive Properties (NF T20-038)	Thermal sensitivity (DIN 1623)		
		Mechanical sensitivity (shock)		
		Mechanical sensitivity (friction)		
	Oxidising Properties (NF T20-035)		Preliminary test[7] mixture of solid with cellulose by weight	
			Train test[7] mixture of solid with cellulose by weight	
	Granulometry			
	Stability in organic solvents and identity of relevant degradation products			
	Dissociation constant			
	Viscosity			
	Particle size distribution	Microscopy examination (OECD TG110) using light or electron microscopy		
			Sieving (OECD TG110)[6,12]	
		Sedimentation (gravitational settling; OECD TG110)		
		Electrical sensing zone (OECD TG110)		
		Phase Doppler anemometry (PDA) – assumes particles are spherical and have a known refractive index		
		Determination of fibre length and diameter distributions (OECD TG110)		
		Cascade impaction		

		Suitable for use with nanomaterials	Concerns over use with nanomaterials	Unsuitable for use with nanomaterials
	Particle size distribution (continued)	Laser scattering/diffraction		
		Rotation drum method		
			Elutriation (OECD TG110)	
			Air jet sieve	
			Cyclone	
A.	Toxicity testing		Histopathological examination in all studies should include electron microscopy	
	Eye Irritation/Corrosion		Acute eye irritation (OECD TG405)[2,7,9]	
	Skin Sensitisation		Guinea pig maximisation test (OECD TG406)[2,7,9]	
			Buehler test (OECD TG406)[2,7,9]	
	Acute Oral Toxicity			Fixed dose procedure (OECD TG420)[2,7,9] – administration of substance by tube (volume required for standard doses)
				Acute toxic class method (OECD TG423)[2,7,9] – administration of substance by tube (volume required for standard doses)
	Acute Inhalation Toxicity		Acute inhalation toxicity (OECD TG403)[2,6,7,9] – range of doses not exceeding 5 % volume of test chamber	
	Acute Dermal Toxicity		Acute dermal toxicity (OECD TG402)[2,7,9]	
	Acute Dermal Irritation/Corrosion		Acute dermal irritation/corrosion (OECD TG404)[2,7,9,10]	

		Suitable for use with nanomaterials	Concerns over use with nanomaterials	Unsuitable for use with nanomaterials
	Repeated Dose (28 days) Toxicity		Oral administration (OECD TG407)[2,7,9]	
			Inhalation administration (OECD TG412)[2,7,9]	
			Dermal administration (OECD TG412)[2,7,9]	
	Sub-Chronic Oral		Repeated dose 90-day in rodents (OECD TG408)[2,5,6(in diet dried form),7] – administration by gavage/diet/drinking water (dependant on material) should ensure that dose is constant	
			Repeated dose 90-day in non-rodents (OECD TG409)[2,5,7] – administration dependant on material and species	
	Sub-chronic dermal		Repeated dose 90-day in rodents (OECD TG411)[2,5,6,7] – solution applied to uncovered skin daily	
	Sub-chronic inhalation		Repeated dose 90-day in rodents (OECD TG413)[2,5,7] – six hours exposure daily	
	Chronic		Chronic Toxicity Test (OECD TG452)[2,5,6,7] daily administration for major proportion of life span by an appropriate route, see subchronic studies	
			Prenatal Developmental Toxicity Study (Teratogenicity, OECD TG414)[2,5,7] – oral administration may not be the most appropriate route, route determined by material properties	

		Suitable for use with nanomaterials	Concerns over use with nanomaterials	Unsuitable for use with nanomaterials
	Chronic (continued)		Carcinogenicity Test (OECD TG451)[2,4,5,6,7] – if substance is made available continuously (e.g. in water or diet) then it should be monitored to ensure a constant exposure level	
			Combined Chronic Toxicity/Carcinogenicity Test (OECD TG453)	
			One-Generation Reproduction Toxicity Test (OECD TG415)[2,4,5,7,11] – normally administered in diet or drinking water	
			Two-Generation Reproduction Toxicity Test (OECD TG416)[2,4,5,7,11] – normally administered in diet or drinking water	
			Toxicokinetics (OECD TG417)[2,7,9,11] – single or repeated doses by appropriate route, human exposure may be by more than one route	
			Neurotoxicity study in rodents (OECD TG 424)[2,4,5,7,11] – oral administration over 28, 90 or 360+ days, inhalation may be more appropriate	
	Mutagenicity	In vitro mammalian chromosome aberration test (OECD TG473) – test substance dissolved or suspended, range of concentrations administered (up to 5 mg/mL or 0.01M)[7]		
		Reverse mutation test using bacteria (OECD TG471) – test substance dissolved or suspended, range of concentrations administered (up to 5 mg/plate)[7]		

		Suitable for use with nanomaterials	Concerns over use with nanomaterials	Unsuitable for use with nanomaterials
	Mutagenicity (continued)		In vivo mammalian chromosome aberration test (OECD TG475) – test substance dissolved or suspended, limit test (2000mg/kg), length of exposure 1 – 14 days[2,7,11]	
			In vivo mammalian erythrocyte micronucleus test (OECD TG474) – test substance dissolved or suspended, limit test (2000mg/kg), length of exposure 1 – 14 days[2,7,11]	
		In vitro gene mutation assay *Saccharomyces cerevisiae* (OECD TG480) – test substance dissolved or suspended, relatively insoluble substances tested up to limit of solubility[2,7]		
		In vitro mitotic recombination assay *Saccharomyces cerevisiae* (OECD TG481) – test substance dissolved or suspended, relatively insoluble substances tested up to limit of solubility[2,7]		
		In vitro mammalian cell mutation assay (OECD TG476) – test substance dissolved or suspended, maximum concentration 5mg/mL or 0.01M[2,7]		
		DNA Damage and Repair – Unscheduled DNA synthesis mammalian cells in vitro (OECD TG482) – test substance dissolved or suspended, range of concentrations (maximum with some cytotoxic effect)[2,7,9]		

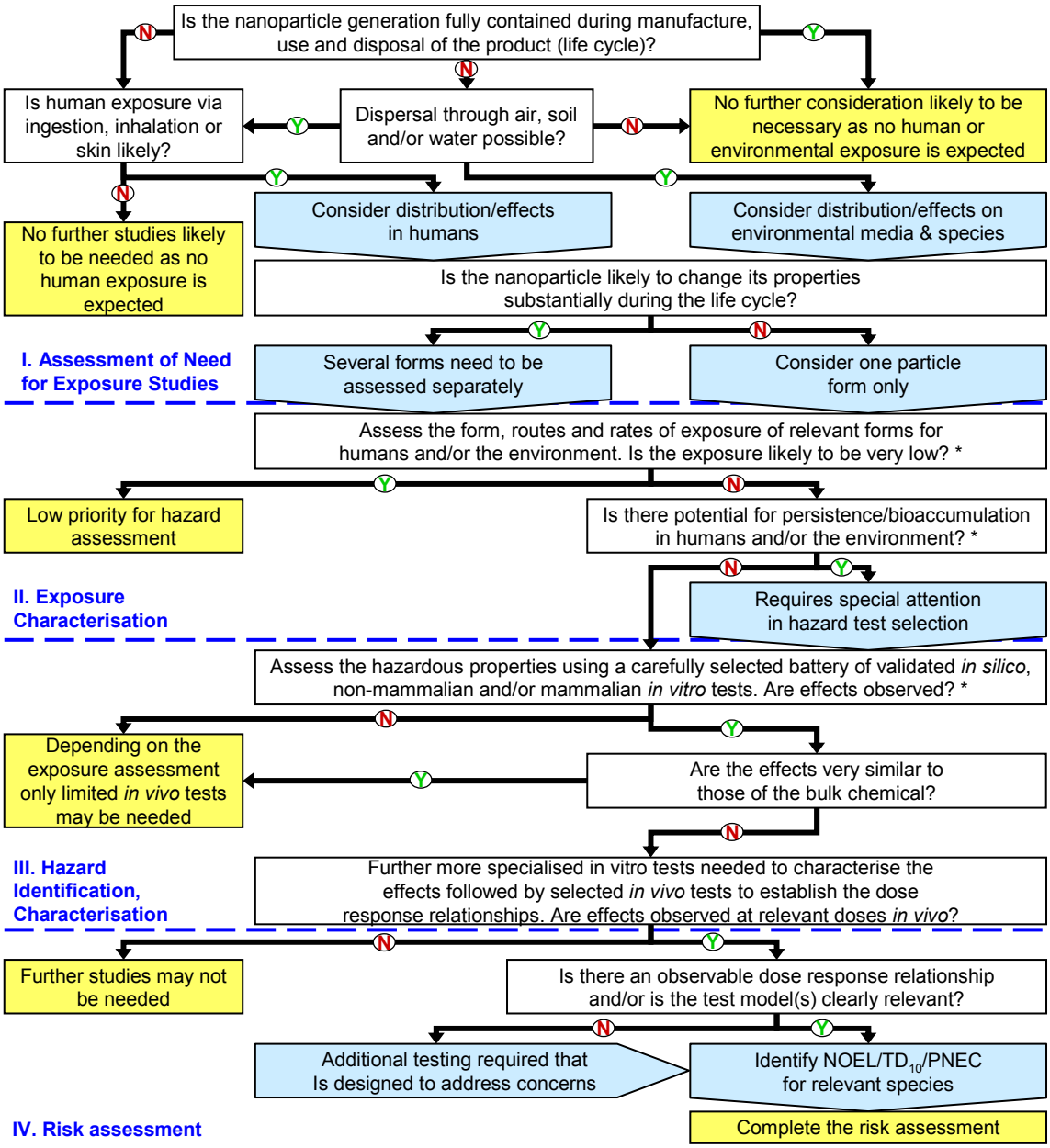
		Suitable for use with nanomaterials	Concerns over use with nanomaterials	Unsuitable for use with nanomaterials
	Mutagenicity (continued)	In vitro sister chromatid exchange assay in mammalian cells (OECD TG479) – test substance dissolved or suspended, range of concentrations (maximum with significant toxic effect, non-soluble tested up to limit of solubility)[2,7,9]		
			Sex linked recessive lethal test in *Drosophila melanogaster* – range of exposures (one either maximum tolerated concentration or indications of toxicity)[2,7,9]	
		In vitro mammalian cell transformation tests –range of exposures (yielding a concentration-related toxic effect), varying duration[2,7,9]		
			Rodent dominant lethal test (OECD TG478) – three dose levels, high dose causing some toxicity, generally single administration[2,4,7,11]	
			Mammalian spermatogonial chromosome aberration test (in vivo, OECD TG483) – range of doses (maximum to no toxicity), limit test of 2000mg/kg body weight/day, one administration[2,7,11]	
		Mouse spot test (in vivo, OECD TG484) – two dose levels (one showing toxicity)		
		Mouse heritable translocation (in vivo, OECD TG485) – appropriate dose and exposure routes used		

[1] visual identification is not possible without microscope
[2] is there enough test substance to enable these tests to be done to a satisfactory standard

[3] elution of nanomaterials may not be possible (interaction with column material)
[4] only suitable for materials that are soluble at concentrations of or greater than 1mg/L
[5] the distinction between a solution and suspension of nanomaterials must be elucidated
[6] concern over nanomaterials becoming airborne during experiment
[7] mixture by weight (another method of determining amount may be more suitable)
[8] not suitable for surface active materials
[9] concern over whether the mentioned endpoints are sufficient – translocation of non-soluble particles should be considered
[10] removal of substance after test
[11] appropriate duration/route
[12] appropriate container for size of material

Appendix 4

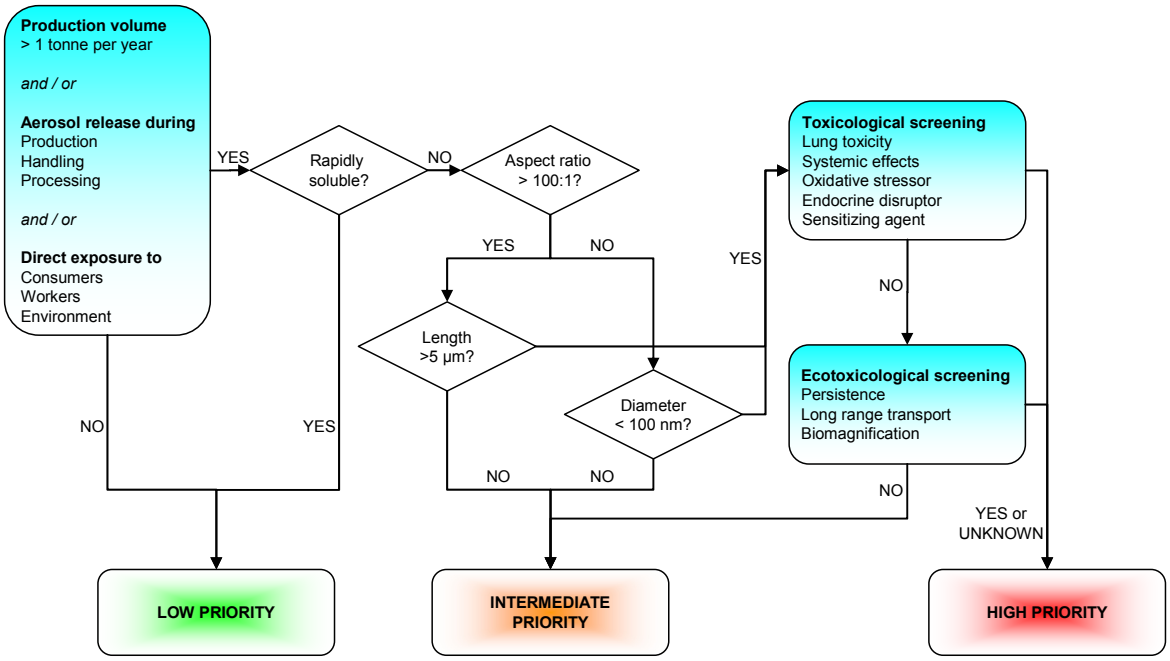
Staged approach for identification of the human and environmental risks from nanomaterials (adopted from SCENIHR, 2007b)



* Compare against the risk assessment for appropriate well studied nanomaterials. Appropriate for benchmarking purposes.

Appendix 5

Proposal for risk assessment of nano-particulate materials (adopted from VDI Technologiezentrum GMbH, 2004)



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Appendix 6

Chemical Safety Report (CSR) on silver (bulk and nano), in official EU template (from p. 87)

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CHEMICAL SAFETY REPORT

Disclaimer: This chemical safety report (CSR) should not be seen as the report of an official chemical safety assessment for silver (bulk and nano). It has been drafted for illustration purposes only, as case study in a report on 'Nanomaterials under REACH', to examine problems that potential registrants may encounter when trying to register a nanomaterial under REACH and to offer suggestions in helping them doing that.

Disclaimer: The data presented in this CSR on bulk silver should not be seen as a complete overview of the toxicity of this compound. No in depth literature search was performed, only readily accessible data were used. The primary information source in this case was an assessment report on silver thiosulphate, drafted within the pesticide framework (CTGB, 2004). For nanosilver the primary information source was a review by Wijnhoven et al. (2009). This was supplemented with relevant information found on nanosilver upon searching the literature for studies that became available since submission of the review.

Disclaimer: In this CSR, data on two soluble silver salts (silver thiosulphate and silver nitrate) have been used as reference substances for Ag^+ toxicity. Use of these data is probably more worst case for metallic silver and nanosilver (both insoluble) than data on less soluble/insoluble silver salts. However, data on the latter were not readily available and were not actively searched for.

Substance Name: Silver (bulk and nano)

EC Number: 231-13-3

CAS Number: 7440-22-4

Registrant's identity: not applicable

Remarks: Distinct Chemical Abstract Service (CAS) numbers are not available for most nanomaterials (exceptions are the CAS numbers of carbon black (CAS no. 1333-86-4) and fullerenes (CAS no. 99685-96-8) which differ from that of carbon (CAS no. 7440-44-0)). CAS numbers are assigned for unique chemical species, with a molecular formula and a unique structure. CAS registry numbers are assigned for chemicals but not for 'materials' per se. Nano-sized silver particles do not (yet?) have a unique CAS number, but only the same CAS as for elemental silver.

When assigning different CAS numbers to nanoforms of a bulk substance it needs to be decided what makes a form different from the bulk, and one nanoform different from the other.

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TABLES

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PART A

1 SUMMARY OF RISK MANAGEMENT MEASURES

2 DECLARATION THAT RISK MANAGEMENT MEASURES ARE IMPLEMENTED

3 DECLARATION THAT RISK MANAGEMENT MEASURES ARE COMMUNICATED

Risk management measures are beyond the scope of this case study.

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PART B

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Table 1: Substance identity.

EC number:	Silver: 231-13-3 (EINECS)
EC name:	Silver
CAS number (EC inventory):	7440-22-4
CAS number:	7440-22-4
CAS name:	Silver
IUPAC name:	Silver
Annex I index number	
Molecular formula:	Ag
Molecular weight range:	107.878

Structural formula: Ag

1.2 Composition of the substance

Table 2: Constituents.

Constituent	Typical concentration	Concentration range	Remarks
Ag	> 99.99 %		

Table 3: Impurities.

Impurities	Typical concentration	Concentration range	Remarks
None have been identified			

Table 4: Additives.

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Remark: The nanosilver particles in the case study are produced in a narrow particle size range, 15 ± 5 nm. Effectively, this means that the size distribution is assumed to be more or less monodisperse. In reality, however, the particle size distribution may be more multidisperse than monodisperse, *i.e.* the mean particle size may still be 15 nm but the distribution is much wider. Since physical, chemical and toxicological properties of nanosilver particles may depend on particle size, it is important to know how to deal with particles outside the intended range of 10 – 20 nm. Are they to be considered as impurities, or as part(s) of the substance, making the substance possibly a multi-constituent substance? The REACH guidance on substance identification is not clear on this.

1.3 Physicochemical properties

Table 5: Overview of physicochemical properties.

Property	Value nanosilver (particles < 100 nm)	Value bulk silver (particles > 100 nm)	Remarks
Physical state at 20°C and 101.3 kPa	Solid	Solid	
Melting/freezing point	**961.93 °C** [1)]	961.93 °C	This is the measured melting point of metallic silver; no data are available for nanosilver. It is likely that this value is also valid for nanosilver.
Boiling point	**2212.0 °C** [1)]	2212.0 °C	This is the boiling point of metallic silver; no data are available for nanosilver. It is likely that this value is also valid for nanosilver.
Relative density	**10.49 g·cm^{-3}** [1)]	10.49 g·cm^{-3}	Nanosilver is a powder
Vapour pressure	Not relevant	Not relevant	
Surface tension	Not relevant	Not relevant	
Water solubility	Insoluble	Insoluble	Nanosilver might form dissolved free silver ions. No information on kinetics of dissolution is available.
Partition coefficient *n*-octanol/water (log value)	Not relevant	Not relevant	
Flash point	Not relevant	Not relevant	
Flammability	Not relevant	Not relevant	
Explosive properties	Explosive	Non explosive	In general, dust explosions may occur when the particle diameter is smaller than 1 – 0.1 mm. The speed of combustion increases with smaller particle sizes with an optimal combustion at particle diameters of approximately 10 – 15 µm (Eckhoff, 2003).
Self-ignition temperature	Not relevant	Not relevant	
Oxidising properties	Not relevant	Not relevant	At best, Ag is a reducing agent
Granulometry	20 ± 5 nm on weight or volume basis, 15 ± 3 nm by area and 8 ± 2 nm by number [2)]	> 100 nm	Please note that it is important to express the unit of the size distribution of nanosilver.

Property	Value nanosilver (particles < 100 nm)	Value bulk silver (particles > 100 nm)	Remarks
Stability in organic solvents and identity of relevant degradation products [3)]	Free Ag^+ ions are formed in aqueous solution	Highly stable	Kinetics of dissolution of nanosilver depends on the composition of the medium and the size distribution of the particles. No information is available. pH and complexing agents like dissolved organic carbon (DOC) and sulphuric ions present in solution will strongly affect dissolution rates, whereas smaller particles will dissolve faster than bigger ones.
Dissociation constant [3)]	Not relevant	Not relevant	
Viscosity [3)]	Not relevant	Not relevant	

1) Values which are assumed to be similar to the corresponding values for bulk silver are indicated in bold: these values are to be considered as best estimates.
2) Granulometry is usually defined as the proportion by weight of particles of different sizes in granular material. Mass and volume are proportional, resulting in a similar mean particle size when granulometry is defined as the proportion by volume. When it is defined as the proportion by surface area or number, the mean size of the particles will be lower, reflecting the fact that the area is inversely proportional to particle volume and to the fact that smaller particles have lower mass.
3) These properties are not required at this tonnage band (10 – 100 tonnes/year), but are required for Annex IX (tonnage band: ≥ 100 tonnes/year).

Remarks: In summary, most basic physicochemical properties of nanosilver particles are similar to those of metallic silver. However, two important physicochemical features are of interest for which additional information is required for a proper risk assessment:

Granulometric aspects. It is essential for a proper risk assessment to have information on the distribution of the main properties that determine the fate and effects of nanosilver. These include (but are not restricted to) particle size distribution, specific surface area, surface charge, and shape of the particles (for instance rods versus ball-shape particles). Various methods are available for measuring these and related parameters, *e.g.* dynamic light scattering, electron microscopy, dynamic imaging (Nanosight), thermal optical transmission.

The kinetics of dissolution of silver nanoparticles as a function of particle properties like size and the composition of the medium (like dissolved organic carbon and pH) as dissolution will yield highly toxic free silver ions. Free silver ions can relatively easily be measured by means of a silver selective electrode.

In all cases, the fate and effect properties of the nanosilver particles basically need to be compared to those of metallic silver and the properties of soluble silver salts like silver nitrate. This is the only way to assess the nano-specific properties of nanosilver as compared to properties of the solid material and dissolved silver ions. In essence, one wants to compare the risk of nanoparticles to the risk posed by solid silver (*i.e.* metallic silver of size in all dimensions > 100 nm). As the ultimate fate of nanosilver is dissolution to yield silver ions, the risks of silver salts need to be included too. In practise, this implies a shift in referencing as initially, solid silver is the reference of choice for comparing physicochemical properties. Solid metal, however, is not transported by advective and diffusive pathways in the environment, and is not toxic to aquatic and terrestrial biota. Given the process of dissolution, silver salts are to be used as a reference for assessing nano-specific impacts on environmental distribution and toxicity. In the latter case, overall toxicity will result from contributions of nanosilver particles and silver ions generated following dissolution of nanosilver particles.

2 MANUFACTURE AND USES

2.1 Manufacture

The case study concerns registration of metallic silver that exists in the nanoform (nanosilver) as well as in the bulk form, with a total tonnage within the 10 – 100 tonnage band.

2.2 Identified uses

For the purpose of this case study, (assumed) uses have been identified for nanosilver only, not for silver in bulk form.

Table 6: Description of identified uses.

Identified use	Sector of Use (SU)	Preparation Category (PC)	Process category (PROC)	Article category (AC)
Production of silver nanoparticles	SU14: Manufacture of basis metals	PC7: Base metals and alloys	For now, worker exposure is beyond the scope of this report.	
Formulation in cleaning products	SU10: Formulation (mixing) of preparation and/or re-packaging	PC35: Washing or cleaning products	For now, worker exposure is beyond the scope of this report.	
Use by general public	SU20: Health services SU21: Private households SU22: Public domain	PC35: Washing or cleaning products		

Remark: The identified uses available under REACH are sufficient to describe the uses of nanomaterials.

2.3 Uses advised against

Beyond the scope of this case study.

3 CLASSIFICATION AND LABELLING

3.1 Classification and labelling in Annex I of Directive 67/548/EEC

Silver is classified:

- for physical-chemical properties: no
- for health effects: no
- for the environment: N; R50-53, S60, S61

For nanosilver, the available toxicity data are too limited to determine C&L for health effects.

In case that the LC/EC_{50} of soluble forms of a metal are determined to be below 100 mg/L, the substance is automatically classified R53, which is in fact the case for nanosilver based on the acute aquatic toxicity results (see chapter 7).

Remarks: In this case the classification and labelling based on silver ions is also applied to the nanoform. This may be considered as a worst-case assumption as the nanosilver is not instantly dissolved. It is recognized, however, that nanosilver itself also contributes to the overall toxicity.

For nanomaterials in general it may by questioned if the trigger values for classification and labelling, based on weight concentrations, are also applicable to nanomaterials or if concentrations expressed in particle numbers or surface area, etc. would be more appropriate.

3.2 Self classification(s) and labelling

Not applicable.

4 ENVIRONMENTAL FATE PROPERTIES

4.1 Degradation

Not relevant for both silver and nanosilver as silver is an element which cannot be degraded.

4.1.1 Abiotic degradation

Two aspects are of importance:

1. The persistence of nanosilver as nanoparticles. This is dependent on the kinetics of hydrolysis/dissolution of the nanoparticles.
2. The long-term persistence is equal to the persistence of silver salts and/or metallic silver as it is expected that in the long term all nanosilver particles will dissolve.

4.1.1.1 Hydrolysis

Not relevant.

4.1.1.2 Phototransformation/photolysis

Not relevant.

4.1.2 Biodegradation

Not relevant.

4.1.2.1 Biodegradation in water

Not relevant.

4.1.2.1.1 Estimated data

Not relevant.

4.1.2.1.2 Screening tests

Not relevant.

4.1.2.1.3 Simulation tests

Not relevant.

4.1.2.2 Biodegradation in sediments

Not relevant.

4.1.2.3 Biodegradation in soil

Not relevant.

4.1.2.4 Summary and discussion on biodegradation

Not relevant.

4.1.3 Summary and discussion on degradation

Not relevant.

Remarks: For nanomaterials in general, it may be assumed that degradation rates are higher when the surface area of particles is increased, *i.e.* the particle size is reduced. However, the influence of shape is unclear and therefore, registrants should show how degradation pathways and rates are altered by this property. This also accounts for composite nanomaterials that consist of combinations of substances (*e.g.* a core of a rare element with a metal coating). Thereupon, the relationship between abiotic degradation rates of nanoparticles and the composition of the medium needs to be quantified by registrants.

Nanoparticles as such may have antibacterial properties due to their small size (Johansen et al., 2008; Navarro et al., 2008b; Neal, 2008), which can for instance be caused by disruption of bacterial membranes, probably by production of reactive oxygen species. Electrostatic interactions may also be important in this respect. Extrapolation/translation from the bulk form to the nano-sized form may then not be possible. Registrants should supply information on this topic.

4.1.4 Environmental distribution

Data needed to assess the environmental distribution of nanosilver particles are not available. In parallel to the behaviour of silver ions in the environment and the general colloidal properties of nanoparticles, it is expected that nanosilver particles will bind strongly to complexing and sorbing agents present in the environmental compartment. Most pronounced sorbing agents are sediment and soil particles, and high partitioning coefficients to these media are expected. Thereupon, strong complexation with universally available complexing agents like dissolved organic carbon and particulate matter (*e.g.* algae) is expected as silver is known to bind strongly to oxygen- and especially sulphur-containing phases.

As with most nanoparticles, nanosilver particles will aggregate. This will further reduce the effective exposure concentrations in aquatic environments, but will increase the concentration of the nanoparticles in sediments. No data are available on the aggregation of nanosilver particles and the kinetics of sedimentation. Finally, stabilization of nanosilver particles by organic material is possible. This will decrease the freely available concentrations of the nanoparticles in the aquatic phase (*i.e.* the concentrations of nanoparticles that are non-complexed and directly capable of interacting with biological surfaces), but will increase the amount of nanosilver particles available for organisms that are capable of releasing them from the stabilized silver-organic matter species.

A potentially important dissipation pathway for nanosilver particles is dissolution and subsequent oxidation to yield ionic silver. Dissolution is a process that basically differs from the process of dissolving of chemicals. Chemicals that dissolve will become hydrated and will yield molecules that are surrounded by water molecules without losing their chemical integrity. Salts that dissolve in water generally yield hydrated cations and anions, *i.e.* charged ions surrounded by water molecules. Metal solids (*i.e.* metals not in salts) do not dissolve as such, independent of the metal solids being present in bulk form or in nanoform. Instead, oxidation of the metal will take place at the surface of the solids. This will yield oxidized metal ions (like Ag^{+}) that are released in the water compartment surrounding

the (bulk or nano) metal solids. Similar to the case of metal salts, the metal ions thus formed will become hydrated and surrounded by water molecules. Metal solids are not distributed in the environment according to equilibrium-based processes and instead will deposit to either sediment or soil instantaneously. Deposited salts will distribute according to the fundamentals of equilibrium partitioning. As the chemical form (silver metal) of nanosilver initially is similar to bulk silver, the nano-specific physicochemical properties of nanosilver are to be compared to the physicochemical properties of silver metal. As dissolution of nanosilver yields dissolved silver ions, the environmental distribution of nanosilver is best compared to the distribution of silver salts. Influences of nano-specific characteristics on nanosilver toxicity are the sum of the contribution of the nanoparticles *sec* and the contribution of the silver ions released. As bulk silver solids are non-toxic, the kinetics of dissolution are of importance in this respect and nano-specific impacts on toxicity need to be compared to the toxicity of silver salts.

The kinetics of dissolution is size and shape dependent and are expected to increase upon decreasing particle size. Thereupon, it is to be expected that the kinetics of dissolution of nanosilver particles depend on the properties of the aquatic medium. Especially pH, dissolved organic carbon (DOC), and silver-complexing ions need to be quantified and identified, as well as the relevant properties of the nanoparticles used, like (time-dependent) shifts in size distribution. No kinetic information is available on these relationships and a comparison with silver particles of size > 100 nm is not possible. Also, it is not possible to use information on dissolution of silver salts as a reference as a fundamentally different process is involved in the dissolution of these salts. Information on dissolution kinetics is especially important for nanosilver particles as free silver ions in general are highly toxic. In case of less toxic free metal ions (like nanoiron particles), and especially in case of metallic nanoparticles that do not dissolve (like nano-TiO_2 or nano-CeO_2), information on dissolution kinetics is less essential or even non-relevant.

Dissolved silver ions are prone to photoreduction leading to a typical metallic-coloured solution due to the presence of metallic silver. In general, however, photoreduction will not affect the risks of either nanosilver or of silver salts or bulk silver. There is no urgency to generate data on this issue.

Remark: As it is unclear which of the processes described above are dominant, registrants should provide information on the behaviour of the nanomaterial under (realistic) worst-case conditions. What is needed is information on dissolution kinetics in dependence of particle properties and properties of the medium and quantitative information on aggregation and complexation of nanosilver particles.

4.1.5 Adsorption/desorption

No sorption data are available for nanosilver. Partition coefficients for ionic silver were summarized and evaluated by Allison and Allison (2005). Based on a literature search, they reported the following values for the log-transformed partition coefficient K_p (units of $L.kg^{-1}$) as determined in a laboratory setting (N = number of observations; no data found for partitioning between Dissolved Organic Carbon and water):

Ag	Soil / Water	Suspended Matter / Water	Sediment / Water
Median	2.6	4.9	3.6
Range	1.0 – 4.5	4.4 – 6.3	2.1 – 5.8
N	21	15	Not given

Remark: Because of the general colloidal properties of nanoparticles, it is expected that nanoparticles in general will bind strongly to complexing and sorbing agents present in the environmental compartment. K_p values may differ between substances in nanoform and dissolved ions, whereas no comparison of sorption properties of nanosilver particles and bulk silver is possible as sorption is not a relevant process for metallic silver in bulk form. Registrants are requested to provide nano-specific information on sorption of nanosilver particles as dependent on the composition of the test medium and the characteristics of the nanosilver particles that determine adsorption/desorption. In principle, standardized OECD and USEPA guidelines are available for quantifying sorption, although some adjustments may be necessary when testing nanomaterials.

4.1.6 Volatilisation

Not relevant.

4.1.7 Distribution modelling

Beyond the scope of this case study.

4.2 Bioaccumulation

4.2.1 Aquatic bioaccumulation

No data on accumulation of nanosilver particles are available.

BCF values for soluble silver (as AgCN, $AgCN(OH)^-$ and $Ag(NH_3)^+$) for fish were determined in brown trout and carp and ranged between 22 and 70 L/kg (CTGB, 2004). These values originated from public literature and were considered to be insufficiently reliable because no validation was provided for the analytical methods for radioactivity measurements in water and fish.

4.2.2 Terrestrial bioaccumulation

No data on accumulation of nanosilver particles are available.

4.2.3 Summary and discussion of bioaccumulation

Remark: At the moment it is not clear whether the current bioaccumulation criteria under REACH are applicable for nanomaterials. The criteria are based on mass concentration, while concentrations based on *e.g.* surface area or particle number may be more relevant for nanomaterials. Also the principle of octanol-water partitioning as surrogate for bioaccumulation is not valid for nanomaterials. This applies for all compartments.

4.3 Secondary poisoning

Not considered in this case study.

5 HUMAN HEALTH HAZARD ASSESSMENT

This CSR is for (metallic) silver, which exists in the nanoform as well as in the bulk form.

As to the bulk form, it can be assumed that all silver compounds (including metallic silver) are transformed (at least in part) to the ionic species, resulting in the silver cation (as measure for dissolved silver species) being the determining factor for *systemic* toxicity. Because of this basic assumption (see also section 4.1.4 for explanation), toxicity data on any silver compound can be used, when expressed as the silver cation. In contrast, for *local* effects in principle only data on metallic silver (or on silver compounds with more or less the same solubility characteristics) can be used.

Available data on silver compounds (in particular silver thiosulphate – STS) have primarily been taken from an assessment report on STS (active substance of Chrysal AVB, a soluble concentrate consisting of 98.7% w/w of STS), drafted within the pesticide framework (CTGB, 2004).

Information on the toxicological implication of the use of silver nanoparticles is limited, as concluded in a review on available data on nanosilver (Wijnhoven et al., 2009). The parts on toxicokinetics, *in vitro* toxicity and human information from that report have been represented here in section 5.1.1, 5.10.1.3 and 5.10.2, respectively, supplemented in section 5.1.1 with an extensive summary of an important toxicokinetic study on nanosilver. As to toxicological endpoints, for nanosilver only data on repeated dose toxicity were available (for the oral and inhalation route), and an *in vivo* micronucleus test. These have been summarised in section 5.6.1.1, 5.6.1.2 and 5.7.1.2, respectively.

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

5.1.1 Non-human information

NANOSILVER

Rat

In a distribution study investigating lungs in particular (Takenaka et al., 2001), female F344 rats were exposed once to ultrafine elemental silver particles (mean diameter 17.1 ± 1.2 nm; generated by spark discharging through an argon atmosphere) by inhalation (whole-chamber) at doses of 0 (clean air) or $3 \cdot 10^6$ particles/cm^3 (133 µg Ag/m^3) for 6 h. For comparison purposes, rats received by intratracheal instillation either 150 µL aqueous solution of 7 µg silver nitrate (4.4 µg Ag) or 150 µL aqueous suspension of 50 µg agglomerated ultrafine elemental silver particles. Two to four treated rats per time point were sacrificed on days 0, 1, 4 and 7 after exposure for morphology analysis of the particles and the lungs and Ag concentration measurements in blood, heart, tracheobronchial and mediastinal lymph nodes (lung associated lymph nodes, LALNs), lung, liver, kidney, nasal cavity (anterior and posterior portion) and brain (including olfactory portion, *i.e.* olfactory bulb + surrounding tissues).

Results: The aerosol inhaled was composed entirely of ultrafine particles that were compact, spherical and electron-dense, with diameters of 4 – 10 nm. In the aqueous suspension, agglomerated particles larger than 100 nm were dominant, but ultrafine particles were also seen. The agglomerates were composed of ultrafine particles that were very similar in shape and size to those inhaled.

In the inhalation study, morphologic analysis showed no accumulation of particle-laden alveolar macrophages or particles in the lung. The Ag concentration and content were highest in the lungs, but decreased rapidly with time, just like Ag concentrations in blood and liver. At day 7, only 4% of the initial burden in the lung remained. At each time point, 9 – 21% of lung content was observed in the liver. Relatively high concentrations were observed in nasal cavity (especially posterior portion) and the LALNs, but Ag was no longer detectable at day 4 and 7, respectively. In the other organs, including brain, low concentrations were detected up until day 4 after exposure. It is notable that after inhalation

of nanosilver, silver reaches the brain, possibly via the olfactory nerve, since the Ag concentration in the olfactory portion was higher than in the rest of the brain.

After a single intratracheal instillation, morphologic analysis revealed accumulation of particles in alveolar macrophages and alveolar walls up to and including day 7 after instillation. In the macrophages, the particles were located in the phagolysosomes, in which both agglomerated and separated ultrafine particles were detectable. Ag analysis showed that 9 – 16 μg of the instilled 50 μg agglomerated ultrafine elemental silver particles was retained in the lungs on day 1, and this content had not decreased by day 7. In contrast, clearance of instilled water-soluble silver nitrate from the lungs was rapid, albeit slower than the clearance of ultrafine elemental silver particles observed in the inhalation study. The liver only contained approximately 3% of the lung content on days 1 – 7 after instillation of agglomerated ultrafine elemental silver particles, whereas after instillation of silver nitrate this was much higher (26 – 56% of lung content). Ag data in other tissues were not reported.

Conclusion: Particle size and the tendency of particles to form agglomerates affect the distribution pathway in the lungs. Alveolar macrophages play a key role in the fate of inhaled fine particles. After intratracheal instillation of agglomerated ultrafine elemental silver particles, approximately 25% of the agglomerates was phagocytized by alveolar macrophages, and remained undissolved in the lungs (in the alveolar macrophages and in alveolar walls) for at least 7 days. After inhalation of ultrafine elemental silver particles, clearance of silver from the lungs was rapid, possibly due to diffusion to blood capillaries, either with or without prior dissolution. This needs further investigation.

Data on ADME for nanosilver (copied from Wijnhoven et al., 2009)

Absorption

Absorption of nano-silver into the human body may occur via inhalatory, oral, and dermal routes of exposure. In kinetic terms, absorption represents the process by which unchanged compounds (e.g., nano-silver) proceed from the site of administration to the central blood circulation and subsequently to the organs (site of measurement).

Lung. The respiratory system represents a major port of entrance for nano-silver. Sprays containing nanosilver are already available on the market, indicating that this is a relevant exposure route. The distribution and disposition of nano-silver in the respiratory tract depends on various factors including particle size and breathing force. In addition, due to the small diameter of the nano-silver, Brownian diffusion also determines deposition, resulting in a deep penetration of nano-silver in the lungs and diffusion to the high lung surface area presented in the alveolar region.

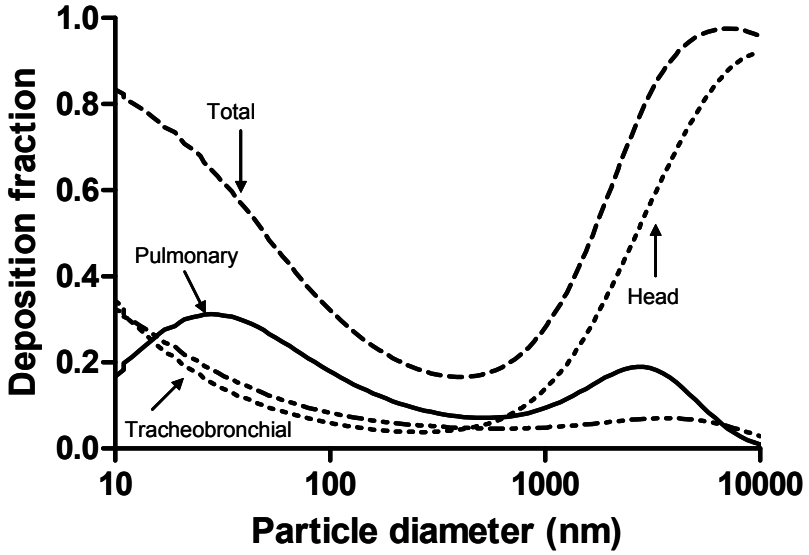
Deposition of (nano)particles can be modeled based on size (International Commission on Radiological Protection [ICRP]) 1994) (Price et al., 2002) by means of mathematical models (Multiple-Path Particle Dosimetry [MPPD] model [Price et al., 2002]).

Figure 1 shows that, according to this model, between 30% and 80% of the inhaled nanoparticles (<100 nm) may be deposited in the respiratory tract. Nanoparticles up to 50 nm are expected to be deposited also in the upper airways due to strong diffusion before transportation into the deep lung (Maynard and Kuempel, 2005).

The deposition of particles in the respiratory tract shown in Figure 1 is modeled with the MPPD model assuming an adult nasal breathing at 7.5 $L.min^{-1}$. Below 10 nm, reliable prediction is not possible, due to model restraints. This model predicts the deposition of inhaled, 'unspecified' particles, i.e., not for nano-silver specific. It was calculated with MPPD v 1.0 (Price et al., 2002).

Two inhalation studies with rats demonstrated that inhalation of nano-silver of ~ 15 nm resulted in the absorption of (nano)silver from the lungs into the systemic circulation (Takenaka et al., 2001; Ji et al., 2007).

Figure 1 Modeled deposition of particles (10 nm – 10 µm) in the respiratory tract.



The olfactory nervous system. An additional absorption route for inhaled nano-silver could be from the olfactory mucosa of the respiratory tract into the central nerve system via the olfactory nerve (Oberdörster et al., 2004). Indeed, inhaled 15 nm nanosilver has been demonstrated to be present in the olfactory nerve and brain of rats after inhalation (Takenaka et al., 2001). This suggests that for inhaled nano-silver, the olfactory nerve represents an additional port of entry into the brain, circumventing the restricted blood-brain barrier (Oberdörster et al., 2004, 2005a,b). However, the relevance and extent of this neuronal absorption route for nano-silver and its presence in humans is unknown (Elder et al., 2006).

Gastrointestinal absorption. The gastrointestinal tract represents an important port of entry for nano-silver at this very moment since specific food products, which are on the market, already contain nano-silver (Maynard and Michelson, 2005). In addition, inhaled nano-silver particles can, in principle, be excreted via the mucociliary escalator and subsequently be ingested into the GI tract. However, there is at the moment no direct evidence confirming this route.

Absorption of silver (both nano-silver and colloidal silver) after oral exposure has been reported. Colloidal silver is characterized as a liquid where silver particles of unknown size, but mainly in nanoscale range, remain in suspension. Absorption of colloidal silver into the systemic circulation is observed to some extent (White et al., 2003; Mirsattari et al., 2004; Chang et al., 2006). A recent oral toxicity study with 60 nm silver nanoparticles, revealed a dose-dependent accumulation of silver content in a broad range of tissues (Kim et al., 2008). This indicates that this accumulated silver content must have been absorbed, reached the systemic circulation, and distributed to other tissues. It remains unclear whether silver ions or the silver nanoparticles were absorbed by the gastrointestinal tract and transported into the body.

Dermal absorption. Dermal exposure represents an important potential absorption route for nano-silver. Antibacterial textiles and wound dressings that contain nano-silver are already on the market. Especially, wound dressings are used on impaired and burned skin and there is direct proof of dermal absorption of (nano-)silver after treatment of burned skin with 15 nm nano-silver coated wound dressings (Acticoat). After one week of local treatment, elevated silver levels in plasma and urine were detected (Trop et al., 2006). This finding was later confirmed by a study with 30 patients treated with Acticoat for small burns (Vlachou et al., 2007). It is, however, unclear from these studies whether silver ions or nano-silver particles were actually transported into the body.

Taken together, it seems that the absorption of nano-silver (and colloidal silver) for various routes of exposure has been identified. However, it remains unclear whether nano-silver particles or silver ions, released from nano-silver at the side of application, were absorbed into the body. Analytical tools for silver measurements do not discriminate between these two types of silver in the kinetic studies reported so far.

Distribution

When nano-silver has passed the barriers (i.e., lung epithelia, intestinal lining, dermis) at the site of entry, the systemic circulation may be reached. Further distribution throughout the entire body may take place via the systemic circulation.

Protein binding. In principle, the binding of plasma proteins influences the ability of a particle to traverse cell membranes and other kinetic parameters (e.g., its distribution and half-life), half life plasma-protein binding. In general for compounds in the blood (e.g., pharmaceuticals), the unbound fraction exhibits the observed effect.

When nano-silver reaches the systemic circulation, the particles can, potentially, interact with plasmaproteins, coagulation factors, platelets and red and white blood cells. An effect of plasma-proteins on the distribution, elimination and toxic potency of nano-silver particles can be expected, similar to described protein (serum albumin) effects on quantum dots (Lovric et al., 2005).

Distribution to organs and tissues. The previously discussed inhalation studies with 15 nm nano-silver particles in rats revealed low, but detectable, concentrations of silver in blood and subsequent distribution to organs including liver, kidney, heart, lymph nodes and brain (Takenaka et al., 2001; Ji et al., 2007). The route by which the brain is reached (via olfactory bulb, via blood brain barrier passage or both) is, however, not known.

Orally ingested colloidal silver resulted in high levels of silver in plasma in one case report, while in another case report, a blue-gray hyperpigmentation of the skin, called argyria was observed. Brown-black granules in a skin biopsy verified colloidal silver as the source of the dyspigmentation (White et al., 2003; Chang et al., 2006). The finding of argyria indicates that ingested colloidal silver is absorbed by the gastrointestinal tract, distributed by the blood to organs and eventually accumulates (partially) in the skin. Kim et al. (2008) found a dose-dependent accumulation of silver content in a broad range of tissues including blood, liver, lungs, kidneys, stomach, testes and brain) in a recent oral toxicity study of 60 nm silver nanoparticles in rats.

Also after dermal exposure of burned skin with nano-silver containing wound dressings (Acticoat) the formation of argyria has been reported (Trop et al., 2006). In this case report, silver plasma concentrations were detected, suggesting dermal absorption, probably of silver, through burned skin and subsequent distribution to tissues such as the skin.

An interesting *in vivo* imaging study with nano-silver in zebra fish embryos revealed nano-silver transport into and out of the zebra fish embryos at each developmental stage studied (Lee et al., 2007, as described above), indicating that (nano-)silver may enter embryonic stages of zebra fish. Increasing concentrations of nano-silver in the exposure medium (in a fraction of particles ranging from 5 – 46 nm) resulted in increased numbers of deformed and dead zebra fish, suggesting that developmental toxicity is highly dependent on the dose of nano-silver (Lee et al., 2007). Although a zebra fish is not readily comparable to humans, this result may indicate that exposure of human fetus cannot be ruled out.

In conclusion, literature on distribution following exposure to nano-silver does not reveal whether silver reaches tissues and organs as nanoparticles. Only silver concentrations were measured in blood and in organs/tissues.

Metabolism

Once nanoparticles are absorbed by the gastrointestinal tract, they will be transported directly to the liver via the portal vein. In general, the liver is able to actively remove compounds from the blood and transform them to chemical forms that can easily be excreted. However, no evidence exists for metabolism of nano-silver by enzymes in the liver and the rest of the body.

It is plausible to assume that nano-silver is able to bind specifically to metallothioneins. Metallothionein proteins that are present in all living cells have a unique structure, depending on their ability to bind metals like zinc and silver. They regulate the cellular metal homeostasis and play a cytoprotective role (Coyle et al., 2002; Lansdown, 2003).

Excretion

Renal elimination is a likely excretion route for nano-silver since it was reported that treatment of burn wounds with nano-silver containing wound dressings (Acticoat) lead to detectable levels of silver in urine of the patient (Trop et al., 2006). It is, however, unclear whether nano-silver or silver ions were excreted in urine.

Uptake of nano-silver by the liver and subsequent excretion in the bile represents another possible excretion route. After inhalation of nano-silver particles of 12.6 – 15.3 nm (Takenaka et al., 2001; Ji et al., 2007), silver was detected in the liver indicating that silver was absorbed. However, excretion via bile and subsequent elimination via feces was not studied in these reports.

SILVER THIOSULPHATE

Rat

For silver thiosulphate (STS), an oral and dermal bioavailability study following single dosing and an oral absorption study following repeated dosing were reported (CTGB, 2004).

Bioavailability study (OECD 417): rats (Wistar; 16 – 24 males/dose), single oral gavage dose of 20 or 200 mg STS/kg bw (vehicle water), single dermal dose ('covered', 8 hrs) of 10 or 100 mg STS/rat (low dose: vehicle water, high dose undiluted), single intravenous dose of 20 mg STS/kg bw (vehicle 5% mannitol in water). Blood and tissues sampled, not excreta. Measurements (of silver) only in blood, not in tissues.

Absorption study (OECD 417): rats (Wistar; 20/sex/dose), oral gavage doses of 20 or 200 mg STS/kg bw/day (vehicle water) for 14 days. Only blood sampled/measured (for silver).

Results: After single oral and dermal administration to rats bioavailability was low. Dermal bioavailability (0 – 2.4%) was somewhat lower than oral bioavailability (1.2 – 4.6%), based on rough estimations. Total absorption was not determined. Maximum blood concentrations were found at 0.5 to 2 hrs after dosing. After intravenous, oral as well as dermal administration, a second blood peak value was seen about 4 – 8 hrs after T_{max} which indicated enterohepatic recirculation. Blood half-life varied widely: about 30 hrs after single intravenous administration of 20 mg STS /kg bw, 6 and 48 hrs after single oral administration of 20 and 200 mg/kg bw STS and 64 hrs after a dermal dose of 200 mg/kg bw STS. During repeated oral administration C_{max} values and AUCs increased indicating accumulation of silver, which is in agreement with the long elimination half-lives.

Female rats possibly have a slower elimination and higher internal exposure compared to male rats. Clearance rate was independent of route and dose: about 15 – 16 mL/h. Distribution volume was 0.7 L after single intravenous administration of 20 mg/kg bw STS, 0.1 and 1 L after single oral doses of 20 and 200 mg/kg bw STS and 1.5 L after a dermal dose of 200 mg/kg bw STS.

Conclusion: Bioavailability after single oral and dermal administration to rats was low (1.2 – 4.6 % and 0 – 2.4 %, respectively). There were indications for enterohepatic recirculation and, upon repeated oral administration, accumulation, consistent with long elimination half-lives. Female rats possibly have a slower elimination and higher internal exposure compared to male rats. No tissue distribution data were

collected. Elimination was also not studied, but there are indications that hepatic route (bile) is an important elimination route.

SILVER/SILVER COMPOUNDS (derived from CTGB, 2004)

Silver compounds can be absorbed via the gastrointestinal tract, the skin, the respiratory tract, and other mucous membranes. The absorption rate is dependent on the route of exposure. Dermal absorption for instance is considered to be low.

Absorbed silver binds to plasma proteins, particularly albumins, transferrins and globulins. In this form it is distributed over the whole body. Therefore, silver can be detected in all kind of tissues of the various organs of both animals and human. Silver mostly forms complexes with sulphur and/or selenium, but can also occur as metallic silver, silver oxide or silver chloride. Silver in the form of selenite or sulphite has a very low solubility, resulting in a low bioavailability. Intracellular it binds to SH-containing proteins, as for instance metallothionein.

In the liver, silver reacts with GSH and the glutathione conjugates are then excreted via bile. More than 90% of the silver is excreted via this route, independently from the form and the route of uptake. The deposition in organs is a result of the precipitation of silver as non-soluble salt, like silver chloride or silver phosphate. These compounds can then be converted in soluble silver sulphite albuminates, which bind to amino-and carboxyl groups in DNA, RNA and proteins. One of the most noticeable effects of chronic exposure to (an-)organic silver and its salts in human is the precipitation of silver in the skin, eyes and other organs accompanied with discoloration (argyria).

5.1.2 Human information

SILVER/SILVER COMPOUNDS (derived from CTGB, 2004)

According to Lyon et al. (J. Environ. Monit., 2002, 4(6): 1034–9) silver concentrations found in neonatal liver samples indicated that silver crosses the human placental barrier. Krachler et al. (Biol. Trace. Elem. Res., 2000, 76(2): 97–112) report presence of highly variable silver concentrations in human milk (according to the authors possibly due to *e.g.* exposure from dental fillings and jewellery). Drasch et al. (J. Trace. Elem. Med. Biol., 1995, 9(2): 82–7) reported that mean silver concentrations in autopsy samples from liver and brain of adult women were approximately twice those of men. In addition, they reported that the concentrations, particularly those in liver, were possibly age dependent. Concentrations in kidney samples were much lower than those in liver and brain.

From public literature it is known that silver accumulates in human skin. Absorbed silver may reach the foetus and may be excreted with mother's milk.

5.1.3 Summary and discussion on toxicokinetics

For nanosilver data are available from a distribution study following single exposure to ultrafine elemental silver particles (mean diameter 17 nm) via inhalation for 6 hrs, as well as following single intratracheal exposure to agglomerated ultrafine elemental silver particles (mostly larger than 100 nm, but also ultrafine particles present). For comparison reasons, silver nitrate was also instilled intratracheally. These data show that (nano-)silver becomes systemically available following single inhalation and intratracheal exposure, possibly in part by oral absorption and by neuronal absorption; the latter because after inhalation silver is found in brain, both in the olfactory and non-olfactory part. However, the form in which it is absorbed and present in tissues (as nanosilver particles, silver ions or both) is not clear, because measurements were on Ag. The clearance rate of silver from the lungs after inhalation exposure to nanosilver particles is much faster than after intratracheal exposure to agglomerated nanosilver, a part of which remained undissolved in alveolar macrophages for at least 7 days following instillation. Instilled water-soluble silver nitrate was also cleared rapidly from the lungs (supporting the theory of rapid solubilization of ultrafine elemental silver in the lung), albeit less

rapidly than nanosilver. A comparison of tissue distribution was not possible, since following instillation data on Ag content in tissues other than lung and liver were not reported. Metabolism, protein binding and excretion following nanosilver administration were not investigated.

Systemic availability of (nano-)silver is also demonstrated in two 28-day rat toxicity studies, with oral and inhalation administration of nanosilver (mean particle size 60 and 12 – 15 nm, respectively), as well as in one 90-day rat inhalation toxicity study (mean size of nanosilver particles 18 – 19 nm). Both exposure routes led to the presence of silver in (amongst others) lungs and brain. Following inhalation exposure, silver was also found in the olfactory bulb, in concentrations higher than in the brain. In humans, topical administration of nanosilver (mean diameter 15 nm) on damaged skin also resulted in absorption, as evidenced by measurable silver concentrations in blood and urine and signs of greyish discoloration.

As to the bulk form, it is known from available data on silver/silver compounds that silver is absorbed following oral, dermal and inhalation administration. It can be assumed that all silver compounds (including metallic silver) are transformed (at least in part) to the ionic species, and that internal exposure is to the silver cation (as measure for dissolved silver species). Absorbed silver binds to plasma proteins and as such is distributed across the body. An important excretion route for silver is via bile. Accumulation of silver can occur in various tissues, *e.g.* skin and eyes, in humans leading to argyria (blue/grey discoloration).

When comparing the nanoform of silver to the bulk form, it is clear that for both forms administration via the oral, dermal and inhalation route results in absorption of silver and distribution throughout the body. For the bulk form, internal exposure will be to the silver ion. However, for the nanoform, where available measurements were on Ag, it is not clear if it is absorbed and present in tissues as nanosilver particles, silver ions or both. But in view of its slow dissolution properties, it is likely to be nanosilver. For the nanoform also data on metabolism and excretion are lacking. Hence, the available data do not allow a conclusion on the kinetics of the nanoform being comparable or different to that of the bulk form.

5.2 Acute toxicity

No acute toxicity studies available on nanosilver, only on silver thiosulphate.

5.2.1 Non-human information

5.2.1.1 Acute toxicity: oral

SILVER THIOSULPHATE

Rat

Method	Results	Remarks	Reference
OECD 423 (limit test)	LD_{50} > 2000 mg/kg bw		CTGB, 2004

Details: rats (Wistar; 3/sex), oral gavage, dose 2000 mg STS/kg bw (no vehicle).

Results: no mortality or clinical signs.

5.2.1.2 Acute toxicity: inhalation

No data available.

5.2.1.3 Acute toxicity: dermal

SILVER THIOSULPHATE

Rat

Method	Results	Remarks	Reference
OECD 402 (limit test)	LD_{50} > 2000 mg/kg bw		CTGB, 2004

Details: rats (Wistar; 5/sex), dermal (occluded), dose 2000 mg STS/kg bw (no vehicle).

Results: no mortality, clinical signs, or skin irritation.

5.2.1.4 Acute toxicity: other routes

No data available.

5.2.2 Human information

No data available.

5.2.3 Summary and discussion of acute toxicity

STS is not considered toxic after acute oral and dermal exposure to rats. According to EU-criteria STS does not need to be classified for acute toxicity (CTGB, 2004).

In the absence of acute toxicity studies with nanosilver, no conclusion can be drawn on its acute toxicity. From the repeated dose toxicity studies available (see section 5.6.1) it can be inferred though, that the highest doses tested in these studies, *i.e.* 1000 mg/kg bw/d orally and $2.85 \cdot 10^6$ particles/cm^3 (515 µg/m^3) via inhalation, do not appear to be acutely toxic since no distinct clinical effects were noted following one or a few doses.

5.3 Irritation

No irritation studies available on nanosilver, only on STS.

5.3.1 Skin

5.3.1.1 Non-human information

SILVER THIOSULPHATE

Rabbit

Method	Results	Remarks	Reference
OECD 404	Not irritating to skin		CTGB, 2004

Details: rabbits (NZW; 3f), dermal (semi-occluded), 4-hr, dose 0.5 ml STS (no vehicle).

Results: Minimal irritation.

5.3.1.2 Human information

No data available.

5.3.2 Eye

5.3.2.1 Non-human information

SILVER THIOSULPHATE

Rabbit

Method	Results	Remarks	Reference
OECD 405	Not irritating to eyes		CTGB, 2004

Details: rabbits (NZW; 3f), ocular (conjunctival sac), dose 0.1 ml STS (no vehicle).

Results: Minimal irritation.

5.3.2.2 Human information

No data available.

5.3.3 Respiratory tract

No data available.

5.3.4 Summary and discussion of irritation

STS is not irritating to skin and eyes of rabbits. According to EU-criteria STS does not need to be classified for skin- or eye irritation (CTGB, 2004).

In the absence of irritation studies with nanosilver, no conclusion can be drawn on its irritating potential. From the repeated dose inhalation toxicity studies available (see section 5.6.1) it can be inferred though, that the highest dose tested in these studies, *i.e.* $2.85 \cdot 10^6$ particles/cm^3 (515 µg/m^3), is not irritating to the respiratory tract when administered for one or a couple of times. From human experience (see section 5.10.2) there are no indications for skin irritation when nanosilver is applied to (intact) skin.

5.4 Corrosivity

STS is not an irritating or corrosive substance. For nanosilver no firm conclusions can be drawn, in the absence of irritation studies.

5.5 Sensitisation

No data available on nanosilver, only on STS.

5.5.1 Skin

5.5.1.1 Non-human information

SILVER THIOSULPHATE

Guinea pig

Method	Results	Remarks	Reference
OECD 406 (GPMT)	Negative		CTGB, 2004

Details: guinea pigs (3/sex control, 5/sex treatment), intradermal induction with 0.1 ml of 10% dilution (vehicle saline), topical induction and challenge with undiluted STS.

Results: No skin reactions.

5.5.1.2 Human information

No data available.

5.5.2 Respiratory system

No data available.

5.5.3 Summary and discussion of sensitisation

STS is not sensitizing in the guinea pig maximization test. According to EU-criteria STS does not need to be classified for sensitization by skin contact (CTGB, 2004).

In the absence of sensitisation studies with nanosilver, no conclusion can be drawn on its sensitizing potential. From human experience (see section 5.10.2) there are no indications for skin sensitisation when nanosilver is applied to (intact) skin.

5.6 Repeated dose toxicity

5.6.1 Non-human information

5.6.1.1 Repeated dose toxicity: oral

NANOSILVER

Rat

Method	Results	Remarks	Reference
OECD 407 (28 days)		Critical effects: liver damage; decreased blood coagulation time in females	Kim et al., 2008

Rats (Sprague-Dawley; 10/sex/dose) received oral gavage doses of 0 (vehicle only), 30, 300 or 1000 mg/kg bw/day silver nanoparticles (average 60 nm, range 52.7 – 70.9 nm) in 0.5 % carboxymethylcellulose for 4 weeks. Observations included clinical signs (daily on weekdays), body weight (once a week), haematology (20 parameters), blood chemistry (22 parameters), organ weights

and microscopy (21 tissues/organs, including brain, lungs, reproductive organs), and determination of silver concentration in blood, lungs, kidneys, brain, stomach, liver and testes.

NB: No information was provided on the magnitude and form/shape of nanoparticles in the dosing solution. Study is stated to be according to OECD 407 (1995), but neurotoxic parameters (*e.g.* functional observational battery, FOB) were not investigated, and histopathology did not include examination of spinal cord, peripheral nerve, bone marrow, lymph nodes, stomach or small or large intestines. It is also unclear whether representative regions of the brain were examined histopathologically.

Results: No distinct clinical effects or effects on body weight were observed, nor effects on organ weights, except for an increase in brain weight in high dose males. Some statistically significant changes in haematology (increases in RBC, Hb, Hc and decrease in blood coagulation time in females at mid and/or high dose, and increase in MCV in high dose males) and biochemistry parameters (increases in ALP and cholesterol in mid- and/or high dose males and females) were observed. Histopathological findings were only observed in livers of male and females rats, consisting of dose-dependent bile-duct hyperplasia, dilatation of the central veins and inflammation (NB: no detailed information available on which dose groups were affected). The silver concentration was dose-dependently increased in blood and all tissues examined, with kidney concentrations in females being 2-fold higher than in males.

Remark: Lack of detailed (histopathological) information prohibited the derivation of a N(L)OAEL.

SILVER THIOSULPHATE

Rat

Method	Results	Remarks	Reference
OECD 408 (90 days)	NOAEL 20 mg/kg bw/d	LOAEL 200 mg/kg bw/day Critical effects: visible pigment deposition; decrease in urine pH in males	CTGB, 2004

Details: rats (Wistar; 10/sex/dose), oral gavage, doses 0, 20, 200, 1000 mg STS/kg bw/day (vehicle tap water) for 3 months. Study included FOB.

Results: A NOAEL of 20 mg/kg bw/day was established, based on the pigment deposits (judged to be undesirable sign of accumulation) in the intestinal tract (mid- and high dose animals) and liver and kidneys (high dose animals) and on decreased urinary pH observed in mid- and high dose males.

Dog

Method	Results	Remarks	Reference
OECD 409 (90 days)	LOAEL 35 mg/kg bw/d	Critical effects: visible pigmentation	CTGB, 2004

Details: dogs (Beagle; 4/sex/dose), oral diet, doses 0, 35, 140, 560 mg STS/kg bw/day (no vehicle) for 3 months.

Results: A NOAEL could not be established because of visible pigmentation (judged to be undesirable sign of accumulation) at 35 mg/kg bw/d (LOAEL) in the intestinal tract. In mid- and high dose animals, pigment deposits were found in the intestinal tract, liver, kidneys, pancreas, mesenteric lymph nodes and adrenals.

5.6.1.2 Repeated dose toxicity: inhalation

NANOSILVER

Rat

Method	Results	Remarks	Reference
OECD 412 (28 days)	NOAEC $1.73 \cdot 10^4$ particles/cm^3 (0.48 µg/m^3)	LOAEC $1.27 \cdot 10^5$ particles/cm^3 (3.48 µg/m^3) Critical effects: liver damage	Ji et al., 2007

In a study according to OECD 412 (1981), rats (Sprague-Dawley; 10/sex/dose) were exposed to silver nanoparticles (ranging from 1.98 – 64.9 nm, average particle size 18 – 19 nm; produced by nanoparticle generator) by inhalation (whole-chamber) at target doses of 0 (fresh-air), $1.2 \cdot 10^4$, $1.2 \cdot 10^5$ or $1.2 \cdot 10^6$ particles/cm^3, 6 h/day, 5 d/wk, for 4 weeks. Observations included clinical signs (daily on weekdays), body weight (once a week), haematology (20 parameters), blood chemistry (22 parameters), organ weights and microscopy (21 tissues/organs, including brain, lungs, reproductive organs), and determination of the silver concentration in blood, lungs, brain, olfactory bulb and liver (in 5/sex/dose).

NB: OECD 412 (original guideline, not updated since 1981) does not include detection of neurotoxic potential, and has limited pathology (*e.g.* no specific examinations of respiratory system).

Results: The actual doses (particles/cm^3) and diameter sizes (geometric mean diameter, GMD), and calculated surface area and mass concentrations were:

Low dose: $1.73 \cdot 10^4$ particles/cm^3, 11.93 ± 0.22 nm, $1.32 \cdot 10^7$ nm^2/cm^3, 0.48 ± 0.25 µg/m^3

Mid dose: $1.27 \cdot 10^5$ particles/cm^3, 12.40 ± 0.15 nm, $9.68 \cdot 10^7$ nm^2/cm^3, 3.48 ± 0.49 µg/m^3

High dose: $1.32 \cdot 10^6$ particles/cm^3, 14.77 ± 0.11 nm, $1.41 \cdot 10^9$ nm^2/cm^3, 61.24 ± 1.52 µg/m^3.

The silver nanoparticles were non-aggregated/agglomerated and assumed to be spherical in shape.

No distinct clinical effects, effects on body weight or organ weight changes were observed. There were only very few small, statistically significant changes in haematology and biochemistry parameters observed, with a slight increase in blood calcium being the only consistent parameter affected in both males and females (high dose only). Histopathological findings were only observed in liver of males (cytoplasmic vacuolization (not dose-related and also in controls) and focal necrosis (high dose only)) and females (cytoplasmic vacuolization (dose-related and also in controls) and focal necrosis (high dose only)). Silver was not detected in blood, but the silver concentration was increased in lungs and olfactory bulb (dose-dependently) and in lungs and brain (high dose only) in both male and female rats, with higher concentrations in the olfactory bulb than in the brain.

Method	Results	Remarks	Reference
OECD 413 (90-days), with special investigation into lung effects	LOAEC $6.64 \cdot 10^5$ particles/cm^3 (48.94 µg/m^3)	Critical effects: lung function changes along with inflammation	Sung et al., 2008 Sung et al., 2009

Rats (Sprague-Dawley; 10/sex/dose) were exposed to silver nanoparticles (ranging from 1.98 – 64.9 nm, average particle size 18 – 19 nm; produced by nanoparticle generator) by inhalation (whole-chamber) at target doses of 0 (fresh-air), $0.6 \cdot 10^6$, $1.4 \cdot 10^6$ or $3.0 \cdot 10^6$ particles/cm^3, 6 h/day, 5 d/wk, for 13 weeks. The target doses were set at approximately ½, 1 and 2 times the high dose in the 28-day inhalation study (Ji et al., 2007). Observations included clinical signs (daily on weekdays), body weight (once a week), lung function tests (once a week after the daily exposure, on 4/sex/dose) by means of plethysmography (parameters included tidal volume (TV), minute volume (MV), respiratory frequency (BPM), inspiration and expiration time (Ti, Te), peak inspiration and peak expiration flow (PIF, PEF)), measurement of differential cell counts (total cells, macrophages, polymorphonuclear cells and lymphocytes) and inflammation parameters (levels of total protein, albumin and LDH) in

bronchoalveolar lavage (BAL) fluid (on same 4 rats/sex/dose after necropsy), and histological examination of the lungs (Sung et al., 2008). Blood chemistry (22 parameters), haematology (20 parameters), organ weights and microscopy (21 organs/ tissues) including lungs, liver, kidney, heart, Harderian gland and prostate, and determination of the silver concentration in lungs, liver kidneys, brain, olfactory bulb and whole blood have been described in another paper (Sung et al., 2009). In addition, evaluation of red blood cell aggregation and a kidney function test has been carried out.

Results: The actual doses (particles/cm^3) and diameter sizes (geometric mean diameter, GMD), and calculated surface area and mass concentrations were:

Low dose: $6.64 \cdot 10^5$ particles/cm^3, 18.12 ± 1.42 nm, $1.08 \cdot 10^9$ nm^2/cm^3, 48.94 ± 0.47 $\mu g/m^3$

Mid dose: $1.43 \cdot 10^6$ particles/cm^3, 18.33 ± 1.12 nm, $2.37 \cdot 10^9$ nm^2/cm^3, 133.19 ± 1.05 $\mu g/m^3$

High dose: $2.85 \cdot 10^6$ particles/cm^3, 18.93 ± 1.59 nm, $6.61 \cdot 10^9$ nm^2/cm^3, 514.78 ± 3.74 $\mu g/m^3$.

The calculated surface area covered by the high dose ($6.61 \cdot 10^9$ nm^2/cm^3) was almost 5-fold greater than that covered by the high dose in the 28-day study ($1.41 \cdot 10^9$ nm^2/cm^3). The silver nanoparticles were spherical in shape and non-aggregated/agglomerated forms with diameters normally distributed from 6 to 55 nm.

Treated male rats showed elevated differential cell counts (not dose-related), with no effect on inflammation markers. In contrast, treated female rats did not show elevated cell counts, but inflammation markers were all increased in the high dose group. As to lung function parameters, TV, MV and PIF were decreased or tended to be decreased in male and female rats. Histopathological examination of the lungs showed significantly increased incidences of mixed cell infiltrate (perivascular) and chronic alveolar inflammation, including granulomatous lesions, alveolar wall thickening and alveolar macrophage accumulation, particularly in high dose rats. Taken together, silver nanoparticle size inhalation induced lung function changes along with inflammation (Sung et al., 2008). No significant differences were found in food consumption between exposed rats and the control group. No distinct clinical effects, effects on body weight or organ weight changes were observed. Also blood haematology and biochemistry parameters were not significantly changed after 90 days of silver exposure. Silver concentrations in lung tissue were significantly increasing with dose and very much higher than in liver, kidney, olfactory bulb, brain and whole blood. Concentrations in olfactory bulb were higher than in brain. Kidney silver concentrations showed a gender difference (higher in females), which could not be verified with the kidney function test. Minimal (but dose related) bild-duct hyperplasia was found in the liver, as well as inflammatory responses in the lung (see above). All other (minimal) histopathological alterations observed in other organs investigated were not exposure-related (Sung et al., 2009).

5.6.1.3 Repeated dose toxicity: dermal

SILVER THIOSULPHATE

Rat

Method	Results	Remarks	Reference
OECD 410 (28 days)	NOAEL systemic 300 mg/kg bw/d	LOAEL 1000 mg/kg bw/day Critical effects: increased ALP, decreased creatinine	CTGB, 2004
	LOAEL local 100 mg/kg bw/d	Critical effects: local irritation	

Details: rats (Wistar; 5/sex/dose), dermal (semi-occlusive), doses 0, 100, 300, 1000 mg STS/kg bw (vehicle water) for 28 days (6 hrs/d, 5 d/wk).

Results: For local effects, no NOAEL could be established because of dermal reactions at all dose levels (LOAEL 100 mg/kg bw/day), first observed after 2 – 3 weeks of exposure. For systemic effects, a NOAEL of 300 mg/kg bw/day was established, based on changes in neutrophils, sodium, total protein and albumin/globulin ratio at the highest dose (possibly secondary to the dermal effects), as well as changes in alkaline phosphatase and creatinine. NB: No information was collected on accumulation in skin and underlying tissues.

5.6.1.4 Repeated dose toxicity: other routes

No data available.

5.6.2 Human information

No data available.

5.6.3 Summary and discussion of repeated dose toxicity

NANOSILVER

Following 28-day oral administration and 28- and 90-day inhalation administration of nanosilver to rats, silver was present in lungs, brain, liver and (after inhalation) in olfactory bulb. Liver damage was the main effect in these studies, but it is to be noted that these studies did not include investigation of neurotoxicity parameters, or detailed histopathological examination of central nervous system and respiratory tract. Inhalation of nanosilver particles for 90-days resulted in lung function changes along with inflammation, whereas no lung damage was seen in the 28-day inhalation study.

SILVER THIOSULPHATE (copied from CTGB, 2004)

The main effects found in the oral studies in rats and dogs were related to accumulation of test compound related material. In the oral rat and dog studies pigmentation was found in parts of the intestine and other organs (liver, pancreas, adrenals). In the rat study, a NOAEL of 20 mg STS/kg bw/day was observed based on pigmentation. In the dog study, a NOAEL could not be established; the LOAEL for pigmentation was 35 mg STS/kg bw/day. These results are in accordance with information from public literature showing that silver may deposit permanently in several tissues in the human body, leading to discoloration (argyria).

No information was collected on accumulation in skin and underlying tissues after dermal administration. It is not known whether no accumulation occurred in those organs in which no pigmentation was visible, but it seems likely that accumulation results only in pigmentation in case concentrations exceed a certain level. Therefore absence of pigmentation does not mean that no accumulation occurred.

SILVER/SILVER COMPOUNDS (derived from CTGB, 2004)

From public literature, it is known that the accumulation of silver in various organs and tissues of the human body may lead to permanent discoloration (argyria), in most cases without accompanying health problems. However, there is also evidence from public literature that at very high exposure, organ toxicity may occur in liver, kidney and nervous system (Schlotzer-Schrehardt et al. (Cornea, 2001, 20(5): 553–7); Ohbo et al. (Psychiatry. Clin. Neurosci., 1996, 50(2): 89–90); Maitre et al. (Ann. Dermatol. Venereol., 2002, 129(2): 217–9)).

In vitro toxicity studies provide evidence for cytotoxicity (metabolic effects, inhibition of proliferation, lethal effects) of silver in fibroblasts and cells of the immune system, *e.g.* in human monocytes (Wataha et al. (J. Oral. Rehabil., 2002, 29(2): 133–9)), human gingival fibroblasts (Locci et al. (J. Biomed.

Mater. Res., 2000, 51(4): 561–8)), human dermal fibroblasts (Hidalgo and Dominguez (Toxicology, 1998, 98(3): 169–79)), human fibroblasts and mast cells (Schedle et al. (J. Dent. Res., 1995, 74(8): 1513–20)), human peripheral T and B lymphocytes and monocytes (Steffensen et al. (Gen. Pharmacol., 1994, 25(8): 1621–33)).

In addition, there are studies suggesting induction by silver of autoimmune responses in mice (Bartova et al. (Neuroendocrinol. Lett., 2003, 24(1–2): 65–7); Johansson et al. (Int. Arch. Allergy. Immunol., 1997, 113(4): 432–43); Hultman et al. (FASEB J., 1994, 8(14): 1183–90)). The risk for humans is not clear, but no extensive literature study on the subject of autoimmune induction by silver in humans was carried out.

5.7 Mutagenicity

5.7.1 Non-human information

5.7.1.1 In vitro data

SILVER THIOSULPHATE

Method	Results	Remarks	Reference
OECD 471 (Ames test)	Negative		CTGB, 2004
OECD 476 (Gene mutation test)	Positive at TK-locus		CTGB, 2004
OECD 473 (Chromosome aberration test)	Negative		CTGB, 2004

Details Ames test: *S. typhimurium* TA98/100/1535/1537, STS (vehicle water), +/- S9.

Results: no induction of point mutations.

Details *in vitro* mammalian gene mutation test: mouse lymphoma L5178Y cells (TK-locus), STS (vehicle growth medium), +/- S9.

Results: increase of large mutant colonies (indication of induction of point mutations) and slightly larger increase of small colonies (which may be due to induction of chromosome aberrations, but is more likely to be caused by the proliferation inhibiting properties of silver). A direct interaction of silver with DNA is not expected. A plausible mechanism for the genotoxicity found in this test is inhibition of enzymes involved in DNA synthesis, due to binding of silver to these proteins. This would implicate an indirect genotoxic mechanism. However, based on the available data, a firm conclusion about the mechanism cannot be drawn.

Details *in vitro* mammalian chromosome aberration test: CHO cells (K-1 line), STS (vehicle growth medium), +/- S9.

Results: no evidence for clastogenicity.

NB: Silver ions may have bound to the proteins in the media and S9 mix used in the three *in vitro* assays, thereby decreasing the exposure of the cells to silver ions and thus decreasing the observed cytotoxicity.

5.7.1.2 In vivo data

NANOSILVER

Method	Results	Remarks	Reference
OECD 474 (Micronucleus test)	Negative		Kim et al., 2008

At the end of a 28-day oral toxicity study with rats (see section 5.6.1.1) the bone marrow was collected for a micronucleus test.

NB: no positive control was included.

Result: The frequency of micronucleated polychromatic erythrocytes (taken as indicator for DNA damage) was slightly but not statistically significantly increased in bone marrow of males and females (up to 1.4x compared to vehicle control). The PCE/(PCE + NCE) ratio (taken as indicator of cytotoxicity) was not affected.

SILVER THIOSULPHATE

Method	Results	Remarks	Reference
OECD 486 (UDS test)	Negative		CTGB, 2004

Details: liver of rats (Wistar; 2 m control, 6 m/dose treatment), oral gavage, single dose of 0, 1000 or 2000 mg/kg bw STS (vehicle: not reported).

Results: no effect on UDS in rat liver cells *in vivo*.

5.7.2 Human information

No data available.

5.7.3 Summary and discussion of mutagenicity

NANOSILVER

Nanosilver did not appear to induce genetic toxicity in rat bone marrow *in vivo*.

SILVER THIOSULPHATE (copied from CTGB, 2004)

STS provided positive results at the TK-locus of mouse lymphoma L5178Y cells. Small mutant colonies increased slightly more than large mutant colonies. The increase of large mutant colonies indicates that point mutations were induced. The slightly larger increase of small colonies may be due to induction of gross chromosome aberrations, but are more likely to be caused by the proliferation inhibiting properties of silver. No evidence for induction of point mutations by STS was found in the Ames test. No evidence for clastogenicity was found an *in vitro* chromosome aberration test in Chinese Hamster Ovary cells. In an *in vivo* rat liver UDS assay no evidence was found for direct interactions of STS with DNA in a relevant target organ under *in vivo* conditions. A direct interaction of silver ions (as in the test solution) or metallic silver (probably one of the forms in which silver is present in the intracellular deposits found in the repeated dose toxicity studies) with DNA would not be expected. Silver ions may inhibit enzymes involved in DNA synthesis due to their protein binding property, which could result in indirect genotoxic effects in the mouse lymphoma assay. However, based on the available data, a firm conclusion about the mechanism cannot be drawn. Taken together, STS is expected to have no direct genotoxic properties *in vivo*.

Thiosulphate is known to inhibit genotoxicity and other toxicity of some compounds, like alkylating agents (possibly by reacting with reactive molecules). It cannot be excluded that under the conditions of the tests (high concentrations of thiosulphate as well as silver ions) there may have been an interaction between effects of thiosulphate and the silver ion effects which is unlikely to occur under physiological conditions (much lower systemic exposure to quickly eliminated thiosulphate, whereas silver accumulates in the tissues).

5.8 Carcinogenicity

No data available.

5.9 Toxicity for reproduction

No data available on nanosilver, only on silver thiosulphate.

5.9.1 Effects on fertility

5.9.1.1 Non-human information

SILVER THIOSULPHATE

Rat oral

Method	Results	Remarks	Reference
OECD 416 (2-generation study)	NOAEL parental tox. 20 mg/kg bw/d	LOAEL 200 mg/kg bw/day Critical effects: visible pigment deposition in F0 females, decreased bw of F1 females	CTGB, 2004
	NOAEL reprotox. 1000 mg/kg bw/d (highest dose tested)	Critical effects: none	

Details: rats (Wistar; 28/sex/dose), oral gavage, doses 0, 20, 200, 1000 mg STS/kg bw/day (vehicle tap water) for 2 generations.

Results: The NOAEL for reproduction toxicity is ≥ 1000 mg/kg bw/day, based on absence of effects on reproduction parameters. The NOAEL for parental toxicity is 20 mg/kg bw/day, based on the effects on maternal body weights (decrease) in the F1 generation and the pigmentation observed in liver, intestine and kidneys of females of the F0 generation. The difference in treatment duration is a plausible explanation for the sex-difference in effect; however there are also indications from the kinetic studies that females may accumulate more silver than males.

5.9.1.2 Human information

No data available.

5.9.2 Developmental toxicity

5.9.2.1 Non-human information

SILVER THIOSULPHATE

Rat oral

Method	Results	Remarks	Reference
OECD 414 (developmental study)	NOAEL maternal tox. 1000 mg/kg bw/d (highest dose tested)	Critical effects: none	CTGB, 2004
	NOAEL embryo/foetal tox. 20 mg/kg bw/d	LOAEL 200 mg/kg bw/d Critical effects: delayed ossification phalanges	

Details: rats (Wistar; 28 f/dose), oral gavage, doses 0, 20, 200, 1000 mg STS/kg bw/day (vehicle tap water) from day 6 – 20 of gestation.

Results: The NOAEL for maternal toxicity is ≥ 1000 mg/kg bw/day, based on absence of effects. The NOAEL for embryo/foetotoxicity is 20 mg/kg bw/day, based on the observed delays in ossification of phalanges at the mid- and high dose. STS induced no irreversible structural effects.

5.9.2.2 Human information

No data available.

5.9.3 Summary and discussion of reproductive toxicity

Up to the highest tested dose (1000 mg STS/kg bw/day) no effects on reproduction and no malformations were found. However, in the two-generation study parental effects (pigment deposition in F0 females and decrease in body weights of the F1 females) were found (NOAEL is 20 mg STS/kg bw/day). In the rat developmental toxicity study, delayed ossification of phalanges in the absence of maternal toxicity was observed. The NOAEL for maternal toxicity was 1000 mg STS/kg bw/day and the NOAEL for embryo/foetotoxicity was 20 mg STS/kg bw/day (CTGB, 2004).

No reproductive toxicity or developmental toxicity studies were available for nanosilver. With respect to fertility, some insight can be gained from the repeated dose toxicity studies available (see section 5.6.1). In these studies, up to the highest dose tested in these studies, *i.e.* 1000 mg/kg bw/d orally and $2.85 \cdot 10^6$ particles/cm^3 (515 µg/m^3) via inhalation, no histopathological effects on the reproductive organs were observed.

5.10 Other effects

5.10.1 Non-human information

5.10.1.1 Neurotoxicity

5.10.1.2 Immunotoxicity

5.10.1.3 Specific investigations: other studies

NANOSILVER

In vitro toxicity (copied from Wijnhoven et al., 2009)

There are various *in vitro* studies on the effects of silver nanoparticles with a size varying between 1 and 100 nm. The uptake of nanoparticles by different cell types has been shown *in vitro* in several, but not all publications (Hussain et al., 2005; Park et al., 2007; Skebo et al., 2007; Suzuki et al., 2007). There is no consensus on the cytotoxicity of nanosilver, however most publications do show reduced cell viability following exposure. Additional toxic effects seen in the *in vitro* studies are glutathione depletion, mitochondrial deviations or destruction and damage to cell membranes.

In vitro exposure of human peripheral blood mononuclear cells (PBMCs) to silver nanoparticles (1 – 2.5 nm, 72 h) resulted in inhibition of phytohemagglutinin (PHA) induced proliferation (at a concentration ≥ 15 ppm) (Shin et al., 2007). Effects on cytokine production were already seen at a low,

non-cytotoxic concentration of 5 ppm. IFNγ and TNF-α production were more severely suppressed than IL-5 production (Shin et al., 2007).

Hussain et al. (2005) evaluated the *in vitro* toxicity of several nanoparticles, including nano-silver (15 and 100 nm) on a rat liver derived cell line (BRL 3A). Following 24 h after exposure the mitochondrial function and membrane integrity (measured as LDH leakage) were significantly decreased (at ≥ 5 $\mu g.ml^{-1}$ and ≥ 10 $\mu g.ml^{-1}$, respectively). LDH leakage was dose dependent and more severe for 100 nm than for 15 nm silver nanoparticles. Visual microscopic evaluation indicated that not all nanoparticles accumulated in the cell, but some remained associated with membranes. All other tested nanoparticles (Fe_3O_4, Al, MoO_3, MnO_2) appeared to be less toxic than nano-silver. The observed cytotoxicity was attributed to be mediated by oxidative stress, as indicated by the detection of GSH depletion, reduced mitochondrial potential, and increased reactive oxygen species (ROS) levels. A similar concentration-dependent cytotoxicity was observed when the effects of the same nano-silver particles on a mouse cell line with spermatogonial stem cell characteristics was studied (Braydich-Stolle et al., 2005). Here, a concentration dependent effect on mitochondrial function, cell viability and membrane integrity (LDH leakage) was seen, albeit at somewhat lower concentrations.

In another study nano-silver particles (~ 30 nm) were classified again to be amongst the most cytotoxic nanoparticles (other nanoparticles were TiO_2, Fe_2O_3, Al_2O_3, ZrO_2, Si_3N_4) when tested on a murine alveolar macrophage cell line, a human alveolar macrophage cell line and epithelial lung cell line (Soto et al., 2005, 2007).

Using a human alveolar epithelial cell line (A549), Park et al. (2007) confirmed that various metallic nanoparticles (Ag, TiO_2, Ni, Zn, Al) induce variable extents of cellular toxicity in a dose dependent manner. However, in this study nano-silver (mean diameter 150 nm, 24 h exposure, and concentrations up to 200 $\mu g.ml^{-1}$) were found to be among the least cytotoxic.

Also neuroendocrine cells were found to be sensitive to the cytotoxic activity of silver nanoparticles (15 nm) (Hussain et al., 2006). Inhibition of dopamine production was only seen at the highest cytotoxic levels, while other known neurotoxic agents (Mn^{2+}, or 40 nm Mn) already reduced neurotransmitter secretion at sub-cytotoxic concentrations.

In contrast, addition of 1.0 % silver nanoparticles (5 – 50 nm) to bone cement, a dose at which antibactericidal activity was seen, did not result in (additional) cytotoxicity towards mouse fibroblasts (L929), or on growth of human osteoblast cell line (hFOB 1.19) (Alt et al., 2004).

Acticoat dressing (wound dressing containing nano-silver) was found to be cytotoxic to primary keratinocytes cultured on a pliable hyaluronate derived membrane (Laserskin) (Lam et al., 2004). Furthermore, reduced mitochondrial metabolism, as well as reduced viability of human keratinocytes and fibroblast cultured on a collagen substrate were detected (cultured skin substitutes, CSS) (Supp et al., 2005). Similar effects (cytotoxicity and disordered morphology) on keratinocytes were reported for extracts of various silver-containing dressings (including Acticoat) (Paddle-Ledinek et al., 2006). Fibroblasts appear to be more sensitive for these effects than keratinocytes (Poon and Burd, 2004). However, when the complexity of the environment increased, e.g., after 3-d culture in collagen lattices, the toxic effect of silver appears to decrease (Poon and Burd, 2004).

Silver colloid becomes adsorbed onto the cell surface (Rospendowski et al., 1992). Treatment of intact human erythrocytes with silver citrate coated colloid (~ 30 nm) induced a large depletion of intracellular glutathione (GSH) (Garner et al., 1994), while bismuth citrate colloid induced oxidation of GSH and no effect of gold colloid on GSH was observed. In lysates of erythrocytes the silver citrate colloid induced oxidation of GSH which was associated with the replacement of the citrate moiety for other naturally occurring species.

Silver nanoparticles (11 nm) were found to enhance the electron-transfer reactivity of myoglobin and peroxidase catalytic activity in phosphate buffered solution (PBS) probably by influencing the heme-group environment (Gan et al., 2004). However, silver nanoparticles might not only help the protein structure to keep its biological activity, but may also act as a conducting wire between the protein and

the electrode that is used for measuring. Whether these are *in vitro* artifacts or whether silver nanoparticles could have a similar effect *in vivo* has not been studied.

5.10.2 Human information

NANOSILVER

Human experience (copied from Wijnhoven et al., 2009)

Clinical observations. Acticoat is a topical wound dressing consisting of a polyethylene mesh coated with nano-silver (average size 15 nm). There is one case report of silver poisoning after the use of Acticoat for treatment of severe burns to the legs (Trop et al., 2006). On day 6 post injury the patient developed a grayish discoloration, complained of being tired and having a lack of appetite. On day 7 silver levels in urine and blood were found to be elevated (28 and 107 $\mu g.kg^{-1}$, respectively). Acticoat was removed and the discoloration of the face gradually faded and liver function test returned to normal values. Elevated blood silver levels were seen seven weeks post injury, but were hardly detectable after 10 months. These observed adverse effects may be associated with the release of Ag^{+} ions from the nano-silver dressing. Absorption of silver from Acticoat was confirmed in 30 patients treated in another study (Vlachou et al., 2007). However, despite measurable amounts of serum silver levels (median 59 $\mu g.L^{-1}$) very limited changes in hematological or biochemical indicators of toxicity associated with the silver absorption were observed.

Furthermore, two case studies have been described of argyria (rare cause of cutaneous discoloration caused by silver deposition) following ingestion of colloidal silver protein (White et al., 2003; Chang et al., 2006). Both single case reports describe the ingestion of colloidal silver solution for treatment of various diseases like 'a cold', dandruff and colonic cancer. Exposure frequency in both studies was estimated to be several times per year during at least 1 year (estimation of total consumption is not known). The source of the patient pigmentation was the colloidal silver rather than other prescribed medication (Chang et al., 2006), and besides elevated serum silver levels no further physical remarks were present following the colloidal silver ingestion (White et al., 2003).

Central nervous system. Epileptic seizures and coma following daily ingestion of colloidal silver for four months were notified in one case report (Mirsattari et al., 2004). The authors suggest that silver caused these signs of irreversible neurological toxicity which eventually lead to death.

Liver. In the case report of Trop et al. (2006), elevated liver enzymes (aspartate amino transferase, alanine aminotransferase and gamma-galactosyl transferase) after the use of Acticoat were reported. Levels returned to normal following cessation of exposure. The patient did not receive any other potentially hepatotoxic medication.

Immune system. Very limited changes in hematological or biochemical indicators of toxicity were associated with the silver absorption from Acticoat in humans (Vlachou et al., 2007), despite measurable amounts of silver in serum. Another case report possibly involving uptake of silver particles is the finding of small electron-dense particles, probably silver nanoparticles, in mast cells following 20 years of local acupuncture (Kakurai et al., 2003). The mast cells showed focal or partial loss of granule content suggesting degranulation (activation) associated with pruritus (itching) and an inflammatory reaction.

Skin. In a moist environment silver is released from the Acticoat dressing (possibly as nanocrystals) and improve microbial control of the wound. Acticoat has been tested in small clinical trials (Tredget et al., 1998; Innes et al., 2001) with contradictory results. No adverse effects were found in the Tredget study, in which silver absorption was not assessed (Tredget et al., 1998). Innes et al. (2001) reported delayed re-epithelization and temporarily worse scars while in another study an increase in re-epithelization was found in meshed skin grafts (Demling and Leslie DeSanti, 2002). An additional case of delayed wound healing has recently been reported (Trop et al., 2006). However, all the studies were small scale and used different controls, thus interstudy comparison is hardly possible.

Ingestion of colloidal silver suspensions led to dyspigmentation of the skin of two patients in two different single case reports (White et al., 2003; Chang et al., 2006).

5.10.3 Summary and discussion

This is dealt with below.

5.11 Summary and discussion on toxicity (5.2–5.10)

For nanosilver, data for repeated dose toxicity were available (for the oral and inhalation route) and an *in vivo* micronucleus test. Oral and inhalatory administration of nanosilver (with mean particle sizes of 60 and 12 – 15 nm, respectively, and doses up to 1000 mg/kg bw/day and $1.32 \cdot 10^6$ particles/cm^3, respectively) to rats for 28 days, as well as inhalatory administration for 90 days (mean particle size of 18 – 19 nm and doses up to $2.85 \cdot 10^6$ particles/cm^3), resulted in the presence of silver in various organs such as liver, lungs, brain and (following inhalation) olfactory bulb. Since measurements were on Ag, it is not clear whether silver is present in these tissues as nanosilver particles, silver ions or both. No histological changes were observed in the lungs following 28 days of nanosilver administration. However, when rats were inhalatory exposed to nanosilver for 90 days, lung function changes along with inflammation were seen. In the latter study the number of particles in the high dose was approximately twice that in the high dose of the 28-day study, but the calculated surface area was almost 5-fold greater. As to other organs than lungs, except for some liver damage no other organ changes were observed in the two 28-day studies and in the 90-day inhalation study. However, it is to be noted that the histological examination of brain, respiratory tract and nervous system was not very extensive, nor did the studies include investigation of neurotoxicity parameters. Clinical effects were monitored, but were absent. No effects were observed in an *in vivo* micronucleus test carried out at the end of a 28-day oral study. Next to these *in vivo* studies, several *in vitro* studies with nanosilver have been described, displaying possible cytotoxic and immunotoxic effects of nanosilver.

With respect to the toxicity of silver in bulk form, it is known that silver compounds are relatively non-toxic, and that the main effect is accumulation of silver in various organs, *e.g.* skin and eyes. In humans the latter results in argyria, in most cases without accompanying health problems. In animal studies with STS, the main effect was visible pigmentation in the gastrointestinal tract, liver and kidneys. This also points to (undesirable) silver accumulation. Except for this pigmentation, no other histological changes were observed in any tissue, including brain. Clinical effects were absent, as well as effects on neurotoxicological parameters (studied in a 90-day oral study with rats).

When comparing the nanoform of silver to the bulk form, it is clear that for both forms repeated administration did not result in clinical effects or other marked toxic effects, despite the presence of silver in various tissues, such as liver, lungs and brain. For the bulk form, the silver concentrations in several tissues were such that they resulted in histologically visible pigmentation. That was not the case for the nanoform, for which it is not clear if it is present in tissues as nanosilver particles, silver ions or both. Furthermore, although there are indications that exposure to nanosilver can lead to damage to the lungs (after inhalation) and to measurable concentrations in the brain (including the olfactory part), neither brain and respiratory tract nor nervous system have been extensively examined histologically. Also no neurotoxicity parameters (*e.g.* FOB) have been investigated, whereas for the bulk form this was done and appeared not affected. When comparing the *in vitro* data on nanosilver and the bulk form, it seems that both may have cytotoxic and immunotoxic effects. A real comparison is however not possible, since none of the investigated parameters have been tested for both compounds in the same study. Hence, the available data do not allow a conclusion on the toxicity of the nanoform being comparable or different to that of the bulk form.

5.12 Derivation of DNEL(s)/DMELs

Beyond the scope of this case study.

NB: The available data would in principle allow the derivation of DNEL(s) for silver in bulk form. For silver in nanoform, it would be a bit more complex, because the available data do not allow a conclusion on the sameness, nor whether route-to-route extrapolation would be justified. Besides, it is uncertain what the appropriate dose metrics would be and whether it is justified to use the standard default values when applying assessment factors in the DNEL derivation.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICOCHEMICAL PROPERTIES

6.1 Explosivity

6.2 Flammability

6.3 Oxidising potential

The human health hazard assessment of physicochemical properties is beyond the scope of this case study.

7 ENVIRONMENTAL HAZARD ASSESSMENT

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity data

7.1.1.1 Fish

7.1.1.1.1 Short-term toxicity of nanosilver and silver nitrate to fish

General description of test design used in Griffitt et al. (2008), which was applied for acute aquatic toxicity testing for fish, *Daphnia* and algae:

For definitive experiments, five concentrations were tested: The estimated median lethal concentration (LC_{50}) from range-finder tests, and 0.6-, 0.36-, 1.67-, and 2.78-fold the estimated LC_{50}. For each test, dosing was performed by preparing stock suspensions of nanometals (except silver) by adding 10 mg of nanometal powder to 10 ml of ultrapure Milli-Q® water (Millipore, Billerica, MA, USA) and then sonicating with a probe sonicator for six half-second pulses at an output of 6 W and 22.5 kHz. Silver nanoparticle (20 – 30 nm) suspensions were prepared in a similar manner, except that a 0.5% sodium citrate solution was used to help stabilize the suspensions. Next, required amounts of nanometal suspensions were added to test chambers by pipetting. The volume of stock solution never exceeded 0.5% of total exposure volume, and addition of sodium citrate had no effect on viability of the organisms being tested. Test solutions were prepared immediately before initiation of the exposures to minimize particle dissolution, aggregation, and sedimentation. Static toxicity tests were performed to generate environmental indices of interest to environmental scientists. For each of the tested nanometals, the LC_{50} was calculated using the trimmed Spearman-Karber method. Concentrations in soluble metal salt exposures were calculated based on the mass of the metal ion rather than the metal salt. For all exposures, control survival was required to exceed 90%; if control survival was less than 90%, the test was repeated.

Table 7: Short term toxicity test with nanosilver nanoparticles or silver nitrate to fish.

Method	Results	Remarks	Reference
ASTM, 2002	LC_{50} 7.07 mg Ag/L (adults) LC_{50} 7.20 mg Ag/L (juveniles)	*Danio rerio* Nanoparticles	Griffitt et al., 2008
ASTM, 2002	LC_{50} 0.0222 mg Ag/L (adults) LC_{50} > 10 mg Ag /L (juveniles)	*Danio rerio* $AgNO_3$	Griffitt et al., 2008
Unknown	LC_{50} 0.0056 mg Ag/L (adults)	*Pimephalus promelas* $AgNO_3$	CTGB, 2004

This fish test with nanosilver particles is 100 to 1000 times less toxic for adult fish than short-term studies with $AgNO_3$ for the same species and an additional species. For juvenile fish the outcome for $AgNO_3$ is equal or less toxic than for nanosilver particles.

7.1.1.1.2 Long-term toxicity to fish

Table 8: Long term toxicity test with silver nitrate to fish.

Method	Results	Remarks	Reference
Unknown	NOEC 0.00024 mg Ag/L (adults)	*Oncorhynchus mykiss* $AgNO_3$	CTGB, 2004

No information for nanosilver is available. Long-term testing is required for fish at the level above 100 tonnes per year according to the REACH requirements as specified in Annex IX.

7.1.1.2 Aquatic invertebrates

7.1.1.2.1 Short-term toxicity to aquatic invertebrates

See for a description of the test design of Griffitt et al. (2008) paragraph 7.1.1.1.1.

Table 9: Short term toxicity test with nanosilver particles and bulk silver to aquatic invertebrates.

Method	Results	Remarks	Reference
ASTM, 2002	LC_{50} 0.04 mg Ag/L (adults)	*Daphnia pulex* Nanoparticles	Griffitt et al., 2008
ASTM, 2002	LC_{50} 0.067 mg Ag/L (neonates)	*Ceriodaphnia dubia* Nanoparticles	Griffitt et al., 2008
ASTM, 2002	LC_{50} 0.008 mg Ag/L (adults)	*Daphnia pulex* $AgNO_3$	Griffitt et al., 2008
ASTM, 2002	LC_{50} 0.16 mg Ag/L (neonates)	*Ceriodaphnia dubia* $AgNO_3$	Griffitt et al., 2008

The results for nanosilver particles and for $AgNO_3$ are comparable.

7.1.1.2.2 Long-term toxicity to aquatic invertebrates

Table 10: Long term toxicity test with bulk silver to aquatic invertebrates.

Method	Results	Remarks	Reference
Unknown	NOEC 0.0016 mg Ag/L (adults)	*Daphnia magna* growth rate $AgNO_3$	CTGB, 2004

No information for nanoscale silver is available. Long-term testing is required for *Daphnia* at the level above 100 tonnes per year according to the REACH requirements as specified in Annex IX.

7.1.1.3 Algae and aquatic plants

See paragraph 7.1.1.1.1 for a description of the test design of Griffitt et al. (2008)

Description of test design of Navarro et al. (2008a):

All experiments were carried out using carbonate coated silver nanoparticles (AgNP) or $AgNO_3$. Carbonate coating serves to maintain AgNP in suspension by avoiding aggregation. Nanoparticle size ranged from 10 to 200 nm, with a median particle diameter of 40 nm. However, in terms of volume, 98 % of the AgNP were within 25 ± 13 nm. The total silver concentration (isotope ^{107}Ag) was measured in acidified solutions (0.1 M HNO_3) by ICP-MS (Element 2 High Resolution Sector Field

ICP-MS; Thermo Finnigan). Reliability of the measurements was controlled using specific water references (National Water Research Institute, Burlington, Canada). Prior to the ICP-MS measurements, AgNP suspensions were digested with HNO_3 in a microwave oven. Recovery of Ag was 88 – 95 %.

Measurement of Ag^+ in the AgNP suspensions by Diffusive Gradients in Thin films (DGT), Ion-Selective Electrode (ISE), and Centrifugal Ultrafiltration. DGT performance to measure Ag^+ was tested (Supporting Information, Tables S2 and S3). Ag^+ concentrations in synthetic media containing AgNP (52 ± 8 nM and 66 ± 9 nM AgNP) were measured by deploying DGT devices. Also, total Ag concentrations were measured at the beginning and at the end of an 8-day period at 25 °C, using ICP-MS. Free Ag^+ was also measured using an Ag-ISE (Metrohm 6.0502.180). The algal (*Chlamydomonas reinhardtii*) photosynthetic yield of the photosystem II in light was measured by fluorometry using a PHYTO-PAM (Heinz Walz GmbH) equipped with an Optical Unit ED-101US/MP.

Table 11: Toxicity test with silver nanoparticles or normal silver to algae and aquatic plants.

Method	Results	Remarks	Reference
ASTM, 2002	EC_{50} 0.19 mg Ag/L	*Pseudokirchneriela subcapitata** Nanoparticles	Griffitt et al., 2008
Unknown	EC_{50} 0.0034 mg Ag/L NOEC 0.00056 mg Ag/L	*Pseudokirchneriela subcapitata* * Silver salts	CTGB, 2004
Not standard (2 hours exposure)	EC_{50} 0.113 mg Ag/L (5 hrs EC_{50} 0.0894 mg Ag/L)	*Chlamydomonas reinhardtii* Nanoparticles	Navarro et al., 2008a
Not standard (2 hours exposure)	EC_{50} 0.02 mg Ag/L	*Chlamydomonas reinhardtii* $AgNO_3$	Navarro et al., 2008a
Not standard (2 hours exposure)	EC_{50} 0.00108 mg Ag^+/L (5 hrs EC_{50} 0.000863 mg Ag^+/L)	*Chlamydomonas reinhardtii* Nanoparticles As function of free Ag^+ at start of experiment	Navarro et al., 2008a

* *Pseudokirchneriela subcapitata* formerly known as *Selenastrum capricornutum*.

On a mass based metric, silver salts are 50 times more toxic for *Pseudokirchneriela subcapitata* than nanosilver particles. $AgNO_3$ is 6 times more toxic for *Chlamydomonas reinhardtii* than nanosilver particles (based on 1 hour exposure 18 times more toxic, not in table). However when compared as a function of the Ag^+ concentration (*i.e.* on mass basis), toxicity of AgNP appeared to be much higher than that of $AgNO_3$ (100 times). The ionic Ag^+ could not fully explain the observed toxicity. Cysteine (added in additional studies), a strong Ag^+ ligand, abolished the inhibitory effects on photosynthesis of both AgNP and Ag^+. Together the results indicate that the interaction of these particles with algae influences the toxicity of AgNP, which is mediated by Ag^+.

7.1.1.4 Sediment organisms

No data available, but also not required at this tonnage band (10t – 100t per year).

7.1.1.5 Other aquatic organisms

No information available, but also not required at this tonnage band (10 – 100 tonnes/year).

Remarks: It is not clear whether results obtained for silver nitrate may also be used for nanosilver particles, because it is unsure whether the toxicity will increase when more silver ions become available over time in the solution. This may also be the case for other metal salts and their nanoforms. For this reason, aquatic toxicity tests, and especially long-term tests, should not be waived, unless the registrant can prove that nanosilver particles are less toxic than dissolved silver or that tests with silver salts (*e.g.* $AgNO_3$) can be considered as a worst case.

Further, it is not certain whether standard toxicity studies focus on the relevant endpoints for nanomaterials. For instance, studies with rats (see section 5.1.1) indicate that nanosilver particles (and possibly nanoparticles in general) of a certain size are able to pass the blood-brain barrier, which may result in effects that differ from those commonly studied in standard toxicity tests.

Next to toxic effects, nanomaterials may also cause a physical stress effect, which was shown for the marine brown alga *Fucus serratus* (Nielsen et al., 2008). Carbon nanotubes affected reproduction as it formed large agglomerates that removed sperm from suspension. Further, processes that were light dependent, such as alignment of the polar axis of zygotes were hampered due to shading of the zygotes by nanoparticle agglomerates. Navarro et al. (2008b) also mention inhibition of photosynthetic activity by shading, reduced gas exchange or cell sinking due to binding of nanoparticles to the cell wall.

Nanoparticles should be tested in ecotoxicity studies as the species in which they occur in the environment (dissolved, stabilized, aggregated, sorbed, etc.) and at (realistic) worst-case conditions regarding the composition of the test medium. For instance, if a reasonable worst-case exposure scenario is that a nanoparticle will always be present in agglomerated form, then this should be the form in which it is tested, so that Predicted Environmental Concentrations (PECs) of the agglomerated form can be compared with PNECs for the same form.

The use of sonication and especially solvents to produce a homogeneous dispersion of nanoparticles at the beginning of ecotoxicity tests may not reflect the behaviour of nanoparticles in the natural environment in which it is possible that agglomerates predominate.

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

7.1.2.1 PNEC water

Table 12: PNEC aquatic.

	Value	Assessment factor	Remarks/Justification
PNEC aqua – freshwater (mg/L) (nanoparticles)	0.04 µg/L	1000	Based on a acute toxicity study with *Daphnia pulex* exposed to nanosilver particles
PNEC aqua – freshwater (mg/L) (Ag^+)	0.024 µg/L	10	Based on a long-term toxicity study with *Oncorhynchus mykiss* exposed to $AgNO_3$
PNEC aqua – marine water (mg/L)	–	–	Beyond the scope of this case study
PNEC aqua – intermittent releases (mg/L)	–	–	Beyond the scope of this case study

NB: These reported PNEC values are based only on the (limited) dataset that was evaluated for this case study. The values for nanosilver particles and silver ions appear to be similar, but it can be questioned whether the assessment factor used for nanosilver can be applied (these assessment values have not been validated specifically for nanomaterials).

7.1.2.2 PNEC sediment

Table 13: PNEC sediment.

	Value	Assessment factor	Remarks/Justification
PNEC sediment (mg/kg dw)	–	–	Beyond the scope of this case study

7.2 Terrestrial compartment

The sole route of exposure for the application assessed here, is via deposition of sewage sludge on soil.

7.2.1 Toxicity data

7.2.1.1 Toxicity to soil macro organisms

No data available, but also not required at this tonnage band (10 – 100 tonnes/year).

7.2.1.2 Toxicity to terrestrial plants

No data available, but also not required at this tonnage band (10 – 100 tonnes/year).

7.2.1.3 Toxicity to soil micro-organisms

No data available, but also not required at this tonnage band (10 – 100 tonnes/year).

7.2.1.4 Toxicity to other terrestrial organisms

No data available, but also not required at this tonnage band (10 – 100 tonnes/year).

7.2.2 Calculation of Predicted No Effect Concentration (PNEC_soil)

Not calculated, because no toxicity data are available.

7.3 Atmospheric compartment

No data available.

7.4 Microbiological activity in sewage treatment systems

No data are available on the effects of nanosilver particles on the microbiological activity in sewage treatment systems. The activated sludge respiration inhibition test is required at the tonnage band of 10 – 100 tonnes/year.

7.4.1 Toxicity to aquatic micro-organisms

In view of the mechanism of action of both nanosilver and silver, and in view of the application as a biocidal agent of both nanosilver and silver salts, information on toxicity of nanosilver is essential as it cannot be ruled out on forehand that nanosilver is equally or less effective than dissolved silver.

7.4.2 PNEC for sewage treatment plant

Not calculated, because no toxicity data are available.

7.5 Non compartment specific effects relevant for the food chain (secondary poisoning)

Because chronic emission into the surface water via the Municipal Sewage Treatment Plant (MSTP) is expected, the manufacturer has to show that there will be no hazard/risk of nanoscale forms after the

use of the products, or that tests with silver salts (*e.g.* $AgNO_3$) can be considered as 'realistic' worst case testing.

Direct primary poisoning is not expected, but indirect exposure via the environment cannot be excluded on forehand.

7.5.1 Toxicity to birds

No long-term data are available for both nanosilver and dissolved silver, but these are also not required at this tonnage band (10 – 100 tonnes/year).

7.5.2 Toxicity to mammals

An over-all DNEL should be derived from the studies performed with mammals within the framework of the human health part.

7.5.3 Calculation of PNEC oral (secondary poisoning)

Table 14: PNEC oral.

	Value	Assessment factor	Remarks/Justification
PNEC oral (mg/kg food)	–	–	Beyond the scope of this case study

7.6 Conclusion on the environmental classification and labelling

Beyond the scope of this case study.

8 PBT AND VPVB ASSESSMENT

8.1 Assessment of PBT/vPvB Properties – Comparison with the Criteria of Annex XIII

8.1.1 Persistence Assessment

According to REACH persistence criteria are not applied to inorganic substances or metabolites. However, the persistence as nanoparticles as such should be clarified by the notifier.

8.1.2 Bioaccumulation Assessment

Beyond the scope of this case study.

8.1.3 Toxicity Assessment

Beyond the scope of this case study.

8.1.4 Summary and overall Conclusions on PBT or vPvB Properties

Beyond the scope of this case study.

8.2 Emission Characterisation

Beyond the scope of this case study.

9 EXPOSURE ASSESSMENT

Environment

Nanosilver is considered to be dangerous to the environment (see section 3.1) and therefore, an exposure scenario should be developed. The relevant tonnage band has to be taken into account, which is 10 – 100 tonnes per year. The first step is to select all relevant Environmental Release Classes (ERCs) for the applications described in section 2.2 to come up with an initial environmental release estimate. The emissions resulting from each ERC is then entered in EUSES (version 2.1) to perform calculations at the local scale and for the total regional emission in order to derive background concentrations.

The general principles of risk assessment of course also apply for nanosilver. It is possible to compare (measured) exposure levels with established safe levels of nanosilver in the environmental compartments (so-called PNEC derivation). However, within the model EUSES many QSPR (quantitative structure-property relationship)-calculations, mainly based on physical-chemical properties of the substance, are used to come up with an estimate for the environmental distribution. From the past it is already known that the usefulness of EUSES for metals is very limited, which will also be the case for nanosilver (although EUSES can be modified). More specifically, for metal ions the concentrations in environmental compartments cannot be predicted from the log K_{ow}. Instead (measured) K_p-values for nanosilver can be used to come up with exposure calculations. At the moment this is not possible as no data on the partitioning coefficients of nanosilver are available. Alternatively in the past a worst-case approach has been used in which it is assumed that 100% of the substance ends up in surface water, and that man is exposed via the environment via drinking water only. This approach can also be applied for nanosilver (see section 9.1.2.4). The outcome of the risk assessment determines if risk management options (RRM) and operation conditions of use (OC) should be included in the dossier as well, to prove that there is no risk involved for the identified exposure scenarios.

Remarks: Among the most important physical-chemical properties the size, water solubility and structural appearance of the substance are the main determining factors for the expectancy of other than traditional narcotic working mechanisms.

To what extent are the specific modes of action of nanomaterials relevant for the environment? Can these be neglected and should the focus be on the soluble forms of the substance?

To what extent can partitioning coefficients for nanomaterials in general be predicted with the log K_{ow} within the exposure model EUSES? Or in other words, to what extent can the fate of nanomaterials be predicted with the conventional models and model descriptors?

9.1 Consumer exposure to nanosilver in bathroom cleaner

For the purpose of this case study, only for nanosilver an exposure assessment has been carried out for its assumed use in a bathroom cleaner (and only for consumers, not for workers), not for silver in bulk form.

9.1.1 Exposure scenario

9.1.1.1 Description of activities and processes covered in the exposure scenario

The exposure scenario is based on the production and use of nanosilver in a bathroom cleaner with 1 % nanosilver particles in a trigger spray. This product is distributed in the environment via the cleaning water and air.

9.1.1.2 Operational conditions related to frequency, duration and amount of use

Table 15: Duration, frequency and amount (Tier 1, ECETOC TRA, revised version for REACH).

Information type	Data field *	Explanation
Number of uses/applications per day/year by one consumer	1 × per day	
Used amount of substance (as such or in preparation) per application	$35 \cdot 10^{-3}$ kg	0.39 g/s × 90 sec
Duration of use per day or per year	4 h exposure	
Annual amount of substance supplied per relevant preparation category(ies)	Not relevant	
Emission days per year related to that preparation category(ies)	Not relevant	

* All values are default values in draft version 1st tier assessments , ECETOC TRA, revised version.

Table 16: Duration, frequency and amount (higher tier, ConsExpo version 4.1).

Information type	Data field *	Explanation
Number of uses/applications per day/year by one consumer	52 × per year	1 × per week
Used amount of substance (as such or in preparation) per application	$35 \cdot 10^{-3}$ kg	0.39 g/s × 90 sec
Duration of use per day or per year	0.42 h/event	25 min
Annual amount of substance supplied per relevant preparation category(ies)	Not relevant	
Emission days per year related to that preparation category(ies)	Not relevant	

* All values are default values in ConsExpo Cleaning Products Fact Sheet (Prud'homme de Lodder et al., 2006).

9.1.1.3 Operational conditions and risk management measures related to product[1] characteristics

Table 17: Characteristics of the substance or preparation.

Information type	Data field	Explanation
Physical state	Liquid in a spray	
For solids: Categorisation of dust grades	Not relevant	
Concentration of substance in preparation	1 %	
Concentration after dilution for use (if relevant)	Not relevant	
Risk management measures related to the design of product		

[1] 'Product' includes substances, preparations and articles.

9.1.1.4 Operational conditions related to available dilution capacity and characteristics of exposed humans

Table 18: Operational conditions related to respiration, skin contact and ingestion (Tier 1, ECETOC TRA, revised version for REACH).

Information type	Data field*	Explanation
Skin contact area	857 cm^2	hands
Mouth contact area	Not relevant	
Respiration volume under conditions of use (= inhalation rate?)	1.37 m^3/h	light activity
Room size and ventilation rate	20 m^3, no ventilation	small room
Body weight	60 kg	adults

* All values are default values in draft version 1st tier assessments , ECETOC TRA, revised version.

Table 19: Operational conditions related to respiration, skin contact and ingestion (higher tier, ConsExpo version 4.1).

Information type	Data field*	Explanation
Skin contact area	860 cm^2	4 x 215 cm^2 = one hand palm
Mouth contact area	–	
Respiration volume under conditions of use (= inhalation rate?)	1.37 m^3/h	light activity
Room size and ventilation rate	10 m^3;, 2 per hour *	bathroom with relatively high ventilation rate
Body weight	60 kg	ConsExpo default value = 60 kg

* All values are default values in ConsExpo Cleaning Products Fact Sheet (Prud'homme de Lodder et al., 2006).

9.1.1.5 Other operational conditions of use

Not relevant.

9.1.1.6 Risk management measures

In the calculation of consumer exposure, risk management measures have not been taken into account. This was considered beyond the scope of the case study.

9.1.1.7 Waste related measures

Not relevant.

9.1.2 Exposure estimation

9.1.2.1 Workers exposure

Not dealt with in this case study.

9.1.2.2 Consumer exposure

Preferably, exposure to substances is assessed using measured data. However, in practice, there is only very little measured data of chemicals released from consumer products. And in case there is measured data, these data only refer to very specific situations. To extrapolate to other exposure situations (*e.g.* larger or smaller room volumes; other amounts) models can be very helpful. Human exposure to substances in consumer products via the dermal or oral route or via inhalation can be modelled with simple or more complex mathematical exposure models. Also in the case of consumer exposure to (nano)silver in a bathroom cleaning product, exposure data were modelled. In the first tier, the revised ECETOC TRA (revised version for REACH) was used, for the higher tier calculation ConsExpo (version 4.1) was used.

9.1.2.2.1 Acute/Short term exposure

First, a tier 1 exposure assessment has been performed (ECETOC TRA revised first tier for REACH) for exposure via inhalation and the dermal route. In this tier 1, the assumption is made that the maximum amount of product ingredient is released in the air to which the consumer is exposed. The relevant product category for this bathroom cleaner with nanosilver is product category C03; 'Cleaners, trigger sprays (all purpose cleaners, sanitary products, glass cleaners)'. The following algorithms are used for the calculation. In these calculations only the values of the product ingredient and bodyweight (adult of child) is variable, other values are fixed values.

Dermal exposure:

PI × CA × FQ × TL × D × 1000 / BW	
PI (product ingredient) [g/g]	**0.01 (1% of nanosilver)**
BW (body weight) [kg)] adult	60 kg
CA (skin contact area) [cm^2] adult	857.5 cm^2
Relevant contact area	hands
FQ (frequency of use)	1 events/day
TL (thickness of layer) [cm]	0.01 (aqueous solutions, other solutions with the viscosity of water (thinners) and non fixed materials (textiles), pastes and viscous materials such as: paints, coatings fillers, putties, lubricants, greases)
D (density) [g/cm^3]	1

Exposure via inhalation:

PI × A × F × FQ × ET × IR × 1000 / V × BW based on body weight	
PI × A × F × 1000 / V based on [mg/m^3]	
PI (product ingredient)	**0.01 (1% of nanosilver)**
BW (body weight) adult	60 kg
A (amount of product for formula)	35 [g / event]
F spray	1 [g/g]
FQ (frequency of use)	1 [events/day]

ET (exposure time)	4 hr
IR (inhalation rate) adult	1.37 [m^3/hr]
V (room volume)	20 m^3

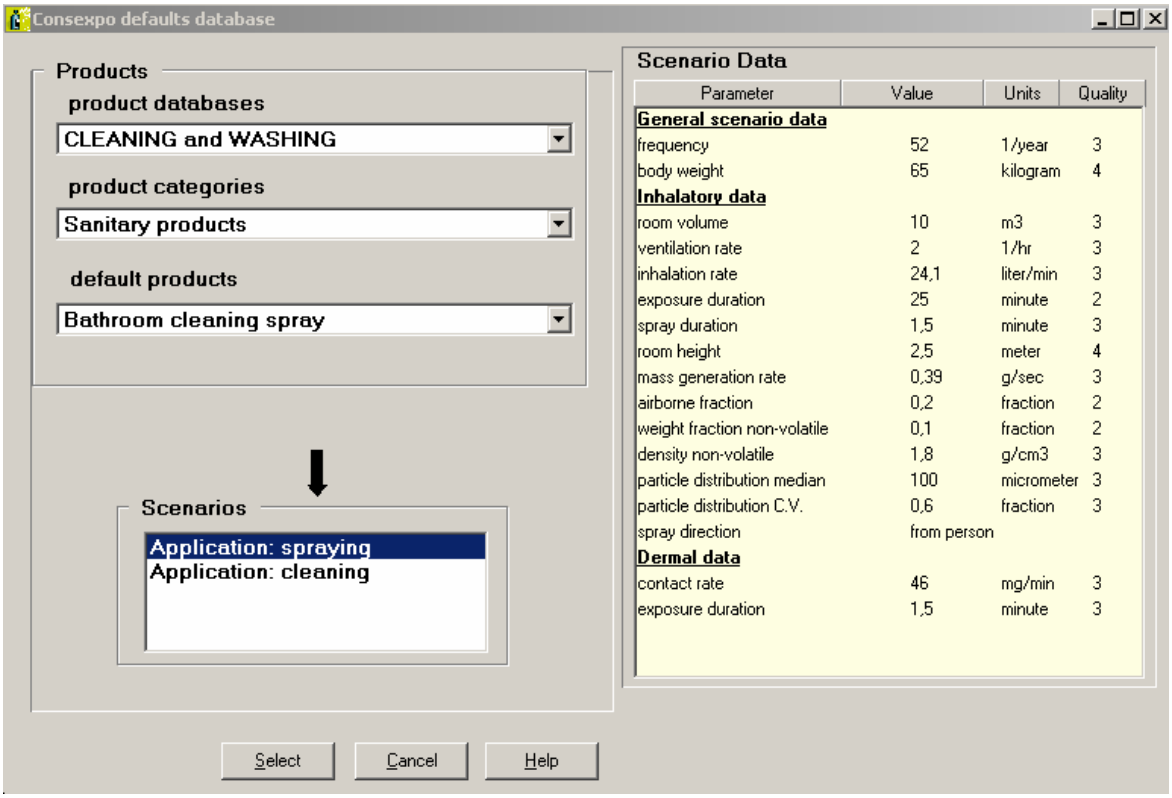
Results for adult (exposure to children is considered not to be significantly different for this use):

Dermal	= 1.43 mg/kg/event
Inhalation on basis bodyweight	= 0.0016 mg/kg/event
Inhalation mg/m^3	= 0.0175 mg/m^3
Total	**= 1.43 mg/kg/event**

NB. The human reference dose is 0.005 mg/kg/day (based on 70 years exposure) (IRIS, US EPA)

Since this dose is remarkably higher than the human reference dose (although they are not directly comparable), a more detailed exposure assessment with the ConsExpo model (version 4.1) has been carried out. Several assumptions are made for the calculation. Again, default values from the ConsExpo Cleaning Products Fact Sheet (Prud'homme de Lodder et al., 2006) are used. From the defaults database the product category sanitary products was selected and as default product a bathroom cleaning spray was chosen.

In the figure below, the inhalation and dermal parameters are presented. These are more complex than the input data (operational conditions in section 9.1.1.2 and 9.1.1.3).



Although no specific data were put into the model for oral exposure, an oral dose is given by the ConsExpo model (based on the fraction of the inhalation dose that has come available via the oral route).

Table 20: Acute exposure concentrations to consumers (modelled via ConsExpo, per event).

Routes of exposure	Estimated Exposure Concentrations		Measured Exposure Concentrations		Explanation / source of measured data
	value	unit	Value	unit	
Oral exposure	0.000317	mg/kg			
Dermal exposure	0.0106	mg/kg			
Inhalation exposure	0.000356	mg/kg			

Integrated point estimates during the event = 0.0109 = **1.1×10^{-2} mg/kg**
NB. The human reference dose is 0.005 mg/kg/day (based on 70 years exposure) (IRIS, US EPA).

Summary of the short-term exposure values.

Table 21: Summary of acute exposure concentrations to consumers.

Routes of exposure	Concentrations	Justification
Oral exposure (in mg/kg bw/d)		
Dermal local exposure (in mg/cm^2)	0.00074	Dermal load/ event
Dermal systemic exposure (in mg/kg bw/d)		
Inhalation exposure (in mg/m^3)	0.000356	Mean event concentration

Remarks: Both models used for calculating consumer exposure are generally used for human exposure assessment of normal, non-nano substances. In this particular case of exposure to nanosilver via inhalation, ConsExpo has calculated the distribution of aerosols in a room from a (trigger)spray. However, this spray model (and its corresponding assumptions such as mass generation rate, airborne fraction, weight fraction non-volatile, density non-volatile, particle distribution median, particle distribution C.V.) has not been validated for nanoparticles. For example, nanoparticles may stay longer airborne. Another assumption in ConsExpo is that particles smaller than 15 µm can be inhaled and are subsequently completely absorbed. For normal substances this is a worst-case assumption. However, for nanoparticles the size-dependent absorption may be an important issue. Therefore, this assumption may need to be refined for nanoparticles.

Also to assess dermal (and oral) exposure in ConsExpo, a fraction should be indicated that passes the barrier of the skin or gastrointestinal tract. However, at the moment it is unclear whether the models that are applied to estimate this fraction for normal non-nano substances are also applicable for nanoparticles. In ConsExpo, dermal absorption can be estimated with a QSAR. This QSAR is already limitedly reliable for non-nano substances, and hence should be validated thoroughly before used for nanoparticles. Until now, the assessment of the validity of an experimentally determined fraction is not part of the ConsExpo model, but still is an important aspect to consider when using a fraction absorbed in an exposure assessment.

More in general, in case of a substance containing nanoparticles, information should be available about the form of the nanoparticles in the consumer product and during exposure. Nanoparticles may agglomerate, aggregate or change form otherwise after or during application in the consumer product. As a consequence, the product may not contain any nanoparticles or nanoparticles with different characteristics than applied in the manufacturing process. Also the conditions during exposure may affect the characteristics of the nanoparticle, for example the conditions in the gastrointestinal tract, in the air or on the skin. It is known that nanoparticles of different size, shape, etc., may display different kinetics and toxicity.

An important issue is that at present little knowledge is available on processes like aggregation and agglomeration, and hence, these processes cannot be modelled (yet). Hence, for the time being, experimental research is required to obtain information about the form of the nanoparticles in the consumer product and during exposure.

9.1.2.2.2 Long-term exposure

No separate long term exposure has been calculated, since the most probable risk is an acute risk per event. However, ConsExpo gives a total chronic dose of $1.5 \cdot 10^{-3}$ mg/kg/day (value of calculated acute dose of $1.1 \cdot 10^{-2}$/7 days).

9.1.2.3 Indirect exposure of humans via the environment (oral)

For man exposed via the environment it is assumed that man is exposed via drinking water only. As a worst case scenario it is assumed that humans are exposed to 2 L/d of untreated river water as drinking water. Based on the PEC_{local} (estimated in section 10) this results in a human exposure of 0.086 µg/kg bw/d (default weight of a human being: 70 kg).

9.1.2.4 Environmental exposure

At the moment insufficient data are available for a proper estimation of environmental exposure to nanosilver. As an alternative a worst-case approach can be applied in which it is assumed that 100% of the substance ends up in surface water as silver ions.

For the present case study it is assumed that 100 tons/year of bathroom cleaner are produced within the EU which contains 1 % of silver. In addition, it is assumed that this cleaner is only used in private use, which is all released into the environment via the sewer system and a municipal waste water treatment plant after which it will all enter the aquatic environmental compartment. For a regional estimation it is assumed that 10% of the 100 tons is released on a regional scale, divided over 365 days.

Using the defaults in EUSES (version 2.1) (*i.e.* local emission is 2 ‰ of the total emission) this results in a local silver emission to the sewage treatment plant (STP) of 0.0548 kg/d (*i.e.* 1 % of the bathroom cleaner). Assuming that none of this silver remains in the STP (sludge) and using the default size of an STP (*i.e.* capacity of 2,000,000 L/d) this results in a concentration of 0.0274 mg nanosilver/L in the STP effluent. For the concentration in the surface water it is assumed that the effluent is diluted 10 times (default in EUSES), resulting in a local emission to surface water of 0.00274 mg/L.

10 RISK CHARACTERISATION

Beyond the scope of this case study.

NB: The focus of the risk assessment under REACH is on risk management. The ultimate aim is to identify and document the conditions of manufacture and use which are needed to control the risks to human health and the environment in an exposure scenario. An exposure scenario therefore includes operational conditions (OCs; *e.g.* duration and frequency of use, amount or concentration of a substance in an activity, process temperature) and risk management measures (RMMs; *e.g.* local exhaust ventilation, wearing certain types of gloves). Risks are regarded controlled when the estimated exposure levels do not exceed the DNELs or PNECs (*i.e.*, ratio exposure/DNEL or exposure/PNEC should be <1). Consequently, when these ratios are above 1, iterations should follow until the ratios become <1.

Besides being beyond the scope of this case study, it was also not possible to demonstrate 'safe use' in the absence of real information on RMMs and OCs. Also, as already indicated in section 5.12, a DNEL has not been derived for nanosilver because that was considered too complex. However, a 'quick and dirty' MOS (margin of safety) approach can be carried out for nanosilver, in order to get an idea of the margins between the estimated exposure and the doses in the toxicity studies with nanosilver at which effects were observed. Comparing the total exposure estimate from ConsExpo (0.011 mg/kg bw, see section 9.1.2.2.1) with the doses tested in the 28-day oral toxicity study (30 – 1000 mg/kg bw, see section 5.6.1.1; all assumed to be effect doses), the margin ranges from 2700 to 90,000. When comparing the inhalation exposure estimate from ConsExpo (0.000356 mg/m^3, for 25 min/day, see section 9.1.2.2.1) with the doses tested in the 28-day and 90-day inhalation toxicity studies (0.48 – 61.24 $\mu g/m^3$ and 48.94 – 514.78 $\mu g/m^3$, respectively, for 6 h/day, see section 5.6.1.2; all assumed to be effect doses), the margins range from 1.3 to 170 and 140 to 1400, respectively.

For the environment a similar 'quick and dirty' approach can be carried out, assuming that all nanosilver dissolves to ionic silver and ends up as such in surface water (compare the exposure scenario in section 9.1.2.4). Based on the estimated local emission to surface water (0.00274 mg/L) the predicted environmental concentration (PEC_{local}) will be 0.0030 mg/L (corrected for regional release). The lowest predicted no effect concentration (PNEC) is 0.000024 mg/L (based on a long-term fish toxicity test with silver nitrate), which indicates that a potential risk is identified (PEC/PNEC >>1). The huge uncertainties and the unrealistic worst-case emission scenario, however, hamper a proper risk evaluation, especially since it was shown that the observed toxicity in algal tests was the result of both Ag^+ ions and suspended particles of nanosilver (Navarro et al., 2008a).

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