SYSTEMATIC EVIDENCE REVIEW FOR DEVELOPING
WHO GUIDELINES ON PROTECTING WORKERS FROM
POTENTIAL RISKS OF MANUFACTURED NANOMATERIALS

Which hazard category should specific nanomaterials or groups of nanomaterials be assigned to and how?

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List of abbreviations

AFM	Atomic Force Microscopy
AgNP	silver nanoparticles
APPT	activated partial thromboplastin time
AuNP	gold nanoparticles
BAL	bronchoalveolar lavage
BET	Brunauer, Emmett and Teller-theory surface to predict bioavailability.
BIAC	Business and Industry Advisory Committee to the OECD
CHO	Chinese hamster ovary cells often used in research
CNI	Carbon Nanotechnologies, Inc.
CNT	carbon nanotubes
DI	Deionized water
ENM	engineered nanomaterials
FWHM	Full width at half maximum, the width of a spectrum curve measured between those points on the y-axis which are half the maximum amplitude.
GHS	UN Globally Harmonized System of Classification and Labelling of Chemicals
GLP	good laboratory practice
IARC	International Agency for Research on Cancer.
ICR mouse	Institute of Cancer Research mouse; special fertile mouse line
ISO	International Organization for Standardization
LC50	Lethal concentration that kills 50% of test sample
LD50	Lethal dose that kills 50% of test sample
LOAEL	low-observed-adverse-effect level
MCA	methylcholanthrene
MMAD	Mass Median Aerodynamic Diameter
MNM	manufactured nanomaterials
MSDS	material safety data sheets
MWCNT	multi-walled carbon nanotubes
ND	not determined
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PT	partial thromboplastin time
RNT	Rosette nanotubes
SD	Sprague Dawley® Rat
SDS	safety data sheet
SWCNTs	single-walled carbon nanotubes
TEM	Transmission Electron Microscope
TG	OECD test guideline
WHO	World Health Organization
WPMN	(OECD) Working Party on Manufactured Nanomaterials
ZnO	zinc oxide

1. Introduction

To address the occupational risks of nanomaterials, the World Health Organization (WHO) is developing guidelines on *Protecting workers from potential risks of manufactured nanomaterials* (WHO/NANOH). These guidelines aim to facilitate improvements in occupational health and safety of workers potentially exposed to nanomaterials in a broad range of manufacturing and social environments. The guidelines will incorporate elements of risk assessment and risk management and contextual issues. They are intended to support policy-makers in Member States with scientific evidence and recommendations for standards and guidance for safe handling of nanomaterials in the workplace. In addition, the guidelines will serve as a basis for the development of an implementation guide containing user-specific guidance and recommendations for target groups, and providing key facts for risk assessment and management.

Hazard is one of the key components in risk assessment and risk management. The hazards of nanomaterials is an area in the nanomaterials risk assessment that has been studied extensively. Currently, numerous papers and reports have been published by academic and research institutions. This systemic review on hazard categorization of nanomaterials describes the current status of nanomaterial hazards to assist in risk assessment and risk management of nanomaterials in the workplace. Proper hazard categorization of nanomaterials will help to classify, label and package nanomaterials and to prepare accurate safety data sheets (SDS). In addition, hazard categorization further facilitates the use of the control banding approach when sufficient information on nanomaterial exposure is not available.

There are many ways to carry out hazard categorization according to regional or local legal requirements. In this systemic review, the hazards of nanomaterials will be categorized according to guidelines outlined in the *UN Globally Harmonized System of Classification and Labelling of Chemicals* (GHS, 6th edition, United Nations 2015). When the manufactured nanomaterial is classified according to the GHS, the specific hazard and category can be identified. The GHS is a widely-supported system that has been developed with the help of authoritative international organizations such as the International Labour Organization, the Organisation for Economic Co-operation and Development (OECD) and the United Nations Economic and Social Council Sub-Committee of Experts on the Transport of Dangerous Goods. The hazard classification used in the GHS has been also standardized by the International Organization for Standardization (ISO) to ISO 11014:2009, *Safety data sheet for chemical products – Content and order of sections*, and further developed into the ISO technical report ISO/TR 13329:2012 on *Nanomaterials – Preparation of material safety data sheet (MSDS)*. The GHS hazard classification approach is being used and implemented in many countries, especially in the field of occupational health and safety.

In this hazard categorization, the hazards of nanomaterials will not be reviewed by searching all the papers relevant to the subject. At the time of our review, more than 10 000 papers had been published in the field of nanomaterial hazard. Thus it is not possible to search all the relevant papers and reports and to review them systemically. The OECD Working Party on Manufactured Nanomaterials (WPMN) was founded in 2006 and started a sponsorship programme on the safety of manufactured nanomaterials. Eleven manufactured nanomaterials that are produced in larger amounts and used in commerce have been subjected to testing and evaluation. The results of this sponsorship programme have been published as comprehensive dossiers that are publicly available. Because this is the most comprehensive source available, we have used these dossiers to systematically review the hazard information collected by the programme.

2. Aim and objectives

The aims of this systematic review are to:

1. Identify the toxicological data needed for hazard identification of the following 11 manufactured nanomaterials (MNMs) in the OECD sponsorship programme dossiers: cerium oxide, dendrimers, fullerenes, gold nanoparticles, multi-walled carbon nanotubes (MWCNTs), nanoclays, silicon dioxide, silver nanoparticles, single-walled carbon nanotubes (SWCNTs), titanium dioxide and zinc oxide.
2. Classify the health hazards for these MNMs, by means of the GHS health classification hazard criteria, into one or more of the following: acute toxicity, skin corrosion/irritation, serious eye damage/eye irritation, respiratory or skin sensitization, germ cell mutagenicity, carcinogenicity, reproductive toxicity, specific target organ toxicity – single exposure, specific target organ toxicity – repeated exposure. Provide data on physical hazard categorization if it is available.

3. Methods

3.1 Inclusion criteria

Population: Manufactured Nanomaterials contained in the published OECD dossiers.

Intervention/exposure: Using the UN Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

Comparator: Not applicable.

Outcome: Classification into one of the following GHS hazard categories: acute toxicity, skin corrosion/irritation, serious eye damage/eye irritation, respiratory or skin sensitization, germ cell mutagenicity, carcinogenicity, reproductive toxicity, specific target organ toxicity – single exposure, specific target organ toxicity – repeated exposure, and aspiration hazard. For each of these hazard categories we classified the MNMs as hazardous, not hazardous or no data available.

Subject: MNM-specific dossiers as developed by the OECD.

3.2 OECD dossiers

The OECD WPMN has tested 11 nanomaterials: Cerium oxide, dendrimers, fullerenes, gold nanoparticles, MWCNTs, nanoclays, silicon dioxide, silver nanoparticles, SWCNTs, titanium dioxide and zinc oxide. The dossiers are available at: http://www.oecd.org/chemicalsafety/nanosafety/dossiers-and-endpoints-testing-programme-manufactured-nanomaterials.htm

3.3 GHS classification criteria

The GHS is based on the following three principles:

1. The GHS covers all hazardous chemicals. The hazard communication elements of the GHS, such as labels or SDS, may vary by product category or stage of the life-cycle. The target audience for the GHS includes consumers, workers in production of materials, in transport and emergency responders.
2. The GHS does not include uniform test methods or promotion of further testing to address adverse health outcomes.
3. In addition to animal data and in vitro tests, human epidemiological data and clinical testing should be considered in application of the GHS.

It is important to note that the GHS is not intended to harmonize risk assessment procedures of risk management decisions, (such as the establishment of a permissible limit for employee exposure), which generally require some risk assessment in addition to hazard classification.

The GHS classifies chemicals into three hazard categories: physical, health and environmental, based on the intrinsic hazardous properties of substances. The health hazards are our primary concern but physical hazards could also be important for developing guidelines on protecting workers from potential risks of manufactured nanomaterials. We will not consider the environmental hazards in this review.

The GHS hazard classification comprises three steps: (a) identification of relevant data regarding the hazards of a substance; (b) review of those data to ascertain the hazards associated with the substance; and (c) a decision on whether the substance will be classified as a hazard and the degree of the hazard, where appropriate, by comparing the data with agreed hazard classification criteria.

3.3.1 Acute toxicity

Acute toxicity refers to those effects occurring following oral or dermal administration of a single dose of a substance, or multiple doses given within 24 hours, or an inhalation exposure of 4 hours.

Table 3.3.1 Acute toxicity hazard categories and exposure routes

EXPOSURE ROUTE	CATEGORY 1	CATEGORY 2	CATEGORY 3	CATEGORY 4	CATEGORY 5
Oral (mg/kg bw)	5	50	300	2000	5000
Dermal (mg/kg bw)	50	200	1000	2000	
Gases (ppmV)	100	500	2500	20000	—
Vapours (mg/L)	0.5	2.0	10	20	
Dusts and mists (mg/L)	0.05	0.5	1.0	5	

Bw: Body Weight; L: litre; ppmV: parts per million per volume.

3.3.2 Skin corrosion/irritation

Skin corrosion is the production of irreversible damage to the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 hours. Skin irritation is the production of reversible damage to the skin following the application of a test substance for up to 4 hours.

Table 3.3.2 Hazard categories and criteria for skin corrosion/irritation

CATEGORY	CRITERIA
Category 1	Destruction and corrosive responses to skin tissue
Category 2	Irritation
Category 3	Mild irritation

3.3.3 Serious eye damage/eye irritation

Serious eye damage is the production of tissue damage in the eye or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application. Eye irritation is the production of change in the eye following the application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application.

Table 3.3.3 Hazard categories and criteria for serious eye damage/irritation

CATEGORY	CRITERIA
Category 1: Serious eye damage/irreversible effects on the eye	In at least one animal, effects on the cornea, iris or conjunctive that are not expected to reverse, or have not fully reversed, within an observation period of normally 21 days; and/or In at least two of three tested animals, a positive response of corneal opacity ≥ 3; and/or iritis ≥ 1.5; calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material.
Category 2: Induce reversible eye irritation	In at least two of three tested animals, a positive response of corneal opacity ≥ 1; and/or iritis ≥ 1; and/or conjunctival redness ≥ 2; and/or conjunctival oedema (chemosis) ≥ 2, calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material, and which fully reverses within an observation period of normally 21 days.

3.3.4 Respiratory and skin sensitizers

A respiratory sensitizer is a substance that will lead to hypersensitivity of the airways following inhalation and a skin sensitizer is a substance that will lead to an allergic response following skin contact.

Table 3.3.4 Hazard categories and criteria for respiratory and skin sensitizers

CATEGORY	CRITERIA
Respiratory sensitizer category 1	A substance is classified as a respiratory sensitizer: a. if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity; and/or b. if there are positive results from an appropriate animal test.
Skin sensitizer category 1	A substance is classified as a skin sensitizer: a. if there is evidence in humans that the substance can lead to sensitization by skin contact in a substantial number of people; or b. if there are positive results from an appropriate animal test.

3.3.5 Germ cell mutagenicity

Germ cell mutagenicity (so-called genetic toxicity or genotoxicity) is concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. Mutagenicity/genotoxicity tests in vitro and in mammalian somatic cells in vivo are considered when classifying substances within this hazard class.

Table 3.3.5 Hazard categories and criteria for germ cell mutagens

CATEGORY	CRITERIA
Category 1	Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans.
Category 2	Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in their germ cells. Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments obtained from: a. somatic cell mutagenicity tests in vivo, in mammals; or b. other in vivo somatic cell genotoxicity.

3.3.6 Carcinogenicity

Carcinogenicity denotes a substance that induces benign and malignant tumours or increases their incidence.

Table 3.3.6 Hazard categories and criteria for carcinogens

CATEGORY	CRITERIA	IARC CLASSIFICATION
Category 1	Known or presumed human carcinogens. The placing of a substance in category 1 is done based on epidemiological and/or animal data.	IARC Group 1 (carcinogenic to humans) IARC Group 2A (probably carcinogenic)
Category 2	Suspected human carcinogens.	IARC Group 2B (possibly carcinogenic)

IARC: International Agency for Research on Cancer.
Note: Most nanomaterials have not been tested for carcinogenicity, except for carbon nanotubes (CNTs).

The International Agency for Research on Cancer (IARC) reviewed the carcinogenicity of CNTs, titanium dioxide and silicon dioxide in Monographs 111 (2017), 93 (2010) and 68 (1997), respectively. Nanoscale titanium dioxide had been tested for carcinogenicity previously and evaluated for carcinogenicity without consideration of uniqueness or terminology of

nanomaterial. Some of the IARC evaluation for CNT and titanium dioxide has been included in this hazard categorization of nanomaterials.

3.3.7 Reproductive toxicity

Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as development toxicity in the offspring.

Table 3.3.7 Hazard categories and criteria for reproductive toxicants

CATEGORY	CRITERIA
Category 1	Known or presumed human reproductive toxicant.
Category 2	Suspected human reproductive toxicant. This category includes substances for which there is some evidence from humans or experimental animals.
Effects on or via lactation	Effects on or via lactation are allocated to a separate single category.

3.3.8 Specific target organ toxicity (single exposure)

Specific target organ toxicity (single exposure) produces specific, non-lethal target organ toxicity arising from a single exposure to a test substance. Other toxicities such as acute toxicity, skin corrosion/irritation, respiratory or skin sensitization, germ cell mutagenicity, carcinogenicity, reproductive toxicity and aspiration are excluded from this category.

Table 3.3.8 Hazard categories and criteria for specific target organ toxicity following single exposure

CATEGORY	CRITERIA
Category 1	Substance that has produced significant toxicity in humans or, based on evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure.
Category 2	Substance that on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to health following single exposure.
Category 3	Transient target organ effects.

3.3.9 Specific target organ toxicity (repeated exposure)

Specific target organ toxicity (repeated exposure) means specific target organ toxicity arising from a repeated exposure. Specific target organ toxicity can occur by oral, dermal or inhalation methods.

Table 3.3.9 Hazard categories and criteria for specific target organ toxicity following repeated exposure

CATEGORY	CRITERIA		
Category 1	Substances that have produced significant toxicity in humans or, based on evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. a. Reliable and good-quality evidence from human cases or epidemiological studies; or, b. Observations from appropriate studies in experimental animals of significant and/or severe toxic effects of relevance to human health. Guidance on dose/concentration values is provided below to be used as part of the weight of evidence evaluation.		
	ROUTE OF EXPOSURE	UNITS	GUIDANCE VALUE
	Oral (rat)	mg/kg bw/d	≤ 10
	Dermal (rat or rabbit)	mg/kg bw/d	≤ 20
	Inhalation (rat) gas	ppm V/6 h/d	≤ 50
	Inhalation (rat) vapour	mg/L/6 h/d	≤ 0.2
	Inhalation (rat) dust/mist/fume	mg/L/6 h/d	≤ 0.02
Category 2	Substances that, based on evidence from studies in experimental animals, can be presumed to have the potential to be harmful to human health following repeated exposure. Placing a substance in category 2 is based on observations from appropriate studies in experimental animals in which significant toxic effects of relevance to human health were produced at generally moderate exposure concentration. Guidance dose/concentration values are provided below to help in classification.		
	ROUTE OF EXPOSURE	UNITS	GUIDANCE VALUE
	Oral (rat)	mg/kg bw/d	10 ⟨ C ≤ 100
	Dermal (rat or rabbit)	mg/kg bw/d	20 ⟨ C ≤ 200
	Inhalation (rat) gas	ppm V/6 h/d	50 ⟨ C ≤ 250
	Inhalation (rat) vapour	mg/L/6 h/d	0.2 C ≤ 1.0
	Inhalation (rat) dust/mist/fume	mg/L/6 h/d	0.02 ⟨ C≤ 0.2

BW: body weight; C:dose/concentration; h/d:hours per day; L: litre; ppmV: parts per million per volume

3.4 Data extraction and management

For each study mentioned in the OECD dossiers for the 11 MNMs, two reviewers independently summarized the toxicological data in the following form.

Table 3.4.1 Data variables to be extracted

	VARIABLE	EXPLANATION/DEFINITION
BACKGROUND	Reference type	OECD WPMN dossier
	Title	Title of dossier
	Bibliographic source	Dossier part number
	Report year	Year dossier was published
	Author	OECD WPMN
STUDY DESIGN AND SETTING	Hazard endpoint	Category of hazard classified by GHS: acute toxicity, skin corrosion/irritation, serious eye damage/eye irritation, respiratory or skin sensitization, germ cell mutagenicity, carcinogenicity, reproductive toxicity, specific target organ toxicity – single exposure and specific target organ toxicity – repeated exposure.
	GLP compliance	Whether the laboratory's test complies with GLP guidelines
	Type of tested material	One of 11 specific nanomaterials
	Test material identified	OECD sponsorship programme identified standard materials for 11 specific nanomaterials
	Test system	Type of animal or type of genetic toxicity in vitro test used
	Test routes of exposure	Oral administration Inhalation Local administration (eye, skin, in vitro) Intratracheal instillation (except for acute toxicity) Intravenous administration.
RESULTS	GHS health hazard classification	Acute toxicity: category 1, category 2, category 3, category 4, category 5, no category or no data. Skin corrosion/irritation: category 1, category 2, category 3, no category or no data. Eye damage/irritation: category 1, category 2, no category or no data. Sensitization: respiratory sensitizer category 1, skin sensitizer category 1, no category for respiratory sensitizer and/or skin sensitizer or no data for respiratory sensitizer and/or skin sensitizer. Repeated specific organ toxicity: category 1, category 2, no category or no data. Genetic toxicity: category 1, category 2 or no category. Carcinogenicity: category 1, category 2, no category or no data. Reproductive toxicity: category1, category 2, effects on or via lactation, no category or no data.

GHS: UN Globally Harmonized System of Classification and Labelling of Chemicals; GLP: good laboratory practice; OECD: Organisation for Economic Co-operation and Development; WPMN: [OECD] Working Party on Manufactured Nanomaterials.

3.5 Assessment of quality of toxicological data

The two reviewers assessed each study for quality. Based on the data quality criteria used by OECD to assess the reliability of toxicological studies (1 = reliable without restriction; 2 = reliable with restriction; 3 = not reliable and 4 = not assignable), they classified the quality of each study as high, medium or low (Table 3.5.1).

The study was classified as high quality if OECD WPMN had rated the data with reliability class 1 or 2 and if the study was conducted in compliance with GLP, based on the OECD test guideline, and/or the study was published in a peer-reviewed journal.

The study was classed as medium quality, if the OECD data rating was 1 or 2 but the study was not conducted in compliance with GLP, based on the OECD test guideline or not based on the OECD test guideline, but it was published in a peer-reviewed journal.

The study was rated as low quality, if the data were classed as OECD reliability class 4 and the study was not in compliance with GLP or the OECD test guideline, and was not published in a peer-reviewed journal.

Table 3.5.1 Data quality criteria

DATA QUALITY	OECD WPMN CATEGORY	GLP COMPLIANCE	BASED ON OECD TEST GUIDELINE	PEER-REVIEW PUBLICATION
High	1 or 2	Yes	Yes	Yes
Medium	1 or 2	No	Yes/no	Yes/no
Low	4	No	No	No

GLP: good laboratory practice; OECD: Organisation for Economic Co-operation and Development; WPMN: [OECD] Working Party on Manufactured Nanomaterials.

3.6 Evidence grade (data synthesis)

The study results can indicate the existence of a hazard or no hazard. A narrative approach to combine the study results was used. In coming to a conclusion, human data of good quality and reliability had precedence. Since human data related to nanomaterials are lacking, positive results from well-conducted animal studies were assessed for robustness and quality in relation to the number of studies.

We also used a single study performed according to good scientific principles and with statistically and biologically significant positive results to justify classification.

Finally, the quality of the evidence was assessed as follows:

On review of each nanomaterial's hazard data, evidence for the existence of a hazard was classified as strong, moderate and low.

- Strong evidence = supported by one or more high-quality study;
- Moderate evidence = supported by one or more medium-quality study and one or more peer-reviewed publication or no peer-reviewed publication;
- Weak evidence = supported by low-quality studies only and no peer-reviewed publications.

If there were contradictory results with the same number of studies within the same data quality classification, the evidence was downgraded by one. If there were contradictory results predominantly within the same data quality classification, the evidence grade remained the same.

3.7 GHS hazard classification of manufactured nanomaterials

The GHS format for recording physical and chemical data on manufactured nanomaterials is shown in Table 3.7.1.

Table 3.7.1 Format for recording physicochemical data on nanomaterials

NANOMATERIAL	DOSSIER NO.	MANUFACTURER	CHARACTERISTICS
Nanomaterials and their assigned name		Company name	Crystalline Colour Primary particle size Agglomerate/aggregate, diameter Specific surface area Zeta potential

The format for recording toxicological data is summarized in Table 3.7.2.

Table 3.7.2 Format for recording toxicological data on nanomaterials

PARTICLE TYPE	NANOMATERIAL TYPE
Species (age and sex)	SD rat/F/8wk
TG/GLP reliability	OECD test guideline number/GLP application
Inclusion criteria	High, medium or low
Route of exposure and dose/exposure concentration	Route of exposure/dose or concentration
Duration of study	Days or months
Findings	LD50, LC50 NOAEL Toxicity findings
Hazard classification	Acute toxicity, irritation, target organ toxicity etc.
Reference other than OECD dossier	Author (year)
Note	No data to classify. No classification

GLP: good laboratory practice; LC50: lethal concentration that kills 50% of test sample; LD50: lethal dose that kills 50% of test sample; NOAEL: no observed adverse effect level; OECD: Organisation for Economic Co-operation and Development; SD rat: Sprague Dawley rat; F, female; TG:Testing Guideline
Note: No data to classify: there are no data to use for classification; no classification: the given data cannot lead to hazard classification.

4. Results

4.1 Fullerenes

Fullerenes evaluated for hazard classification are listed in Table 4.1.1.

Fullerenes were a reference material, or alternative reference material, for the OECD sponsorship programme for the testing of manufactured nanomaterials. Their extensive physicochemical characterization data can be found in the following published dossiers (http://www.oecd.org/chemicalsafety/nanosafety/fullerenes-c60-manufacturednanomaterial.htm).

Table 4.1.1 Physicochemical properties of fullerenes

NANOMATERIAL	DOSSIER NO.	MANUFACTURER	CHARACTERISTICS
Fullerene C60	No 48. ENV/JM/MONO(2015)11/PART1, PART2	Nano purple (Frontier Carbon Corporation) C60 MER Corp.	Crystalline Black Primary particle size 20 nm–30 nm Agglomerate/aggregate diameter > 500 nm Specific surface area 0.87 m^2/g Zeta potential ca 20 mV

4.1.1 Acute toxicity of fullerenes

The acute oral and inhalation toxicity of fullerenes has been studied in experimental animals. An acute oral study conducted for fullerene based on Technical Guideline (TG) 474 showed no mortality at maximum experiment concentration and no acute toxicity hazard was assigned for fullerene.

4.1.2 Dermal and eye irritation/corrosion of fullerenes

Studies of dermal and eye irritation/corrosion caused by fullerenes, based on TG 404 and TG 405 respectively, showed that they did not cause irritation and corrosion to either skin or eyes. Hence, fullerene was not assigned to a hazard categorization.

4.1.3 Skin sensitization of fullerenes

Skin sensitization studies based on TG406 for fullerenes showed a slightly irritating effect, but fullerenes were still not assigned to a hazard categorization.

4.1.4 Specific target organ toxicity arising from repeated exposure to fullerenes

Fullerene has been tested subacutely based on TG 407. Although there was a decrease in albumin in males and an increase of total protein in females, there was no histopathological change. The NOAEL (no observed adverse effect level) of repeated oral dose toxicity of the fullerene was considered to be 1000 mg/kg bw/day. It is very difficult to categorize the specific target organ toxicity. Fullerene was tested for repeated dose toxicity through inhalation subacutely. There was only a slight inflammatory response in the lungs at test level (0.12 mg/m^2) of fullerene.

4.1.5 Genetic toxicity of fullerenes

Fullerenes tested for in vitro genotoxicity based on TG 471 were negative for bacterial reverse mutation assay, with or without the presence of a metabolic system. Chromosomal aberration tests conducted for fullerenes were negative.

In vivo genotoxic studies for fullerenes, based on the TG 474 mammalian erythrocyte micronucleus test, were negative. For the in vivo comet assay, fullerenes also showed a negative response.

Table 4.1.2 Summary of genotoxicity of fullerenes

BACTERIAL REVERSE MUTATION ASSAY	CHROMOSOMAL ABERRATION	IN VITRO MAMMALIAN CELL MICRONUCLEUS TEST	IN VITRO SINGLE CELL GEL/COMET ASSAY IN MAMMALIAN CELLS FOR DETECTION OF DNA DAMAGE	IN VIVO MAMMALIAN ERYTHROCYTE MICRONUCLEUS TEST	DNA DAMAGE AND/OR REPAIR, MOUSE SPOT TEST
Negative	Negative	No data	No data	Negative	Negative

Table 4.1.3 Data quality of fullerene studies

HEALTH HAZARDS	NO. OF STUDIES	NO. OF HIGH QUALITY (HAZARD CATEGORY)	NO. OF MEDIUM QUALITY (HAZARD CATEGORY)	NO. OF LOW QUALITY (HAZARD CATEGORY)	EVIDENCE GRADE
Acute toxicity	1	1 (No classification)	NA	NA	Strong
Skin corrosion/irritation	2	NA	2 (All no classification)	NA	Moderate
Serious eye damage/eye irritation	1	NA	1 (No classification	NA	Moderate
Respiratory or skin sensitization	2	NA	2 (All no classification)	NA	Moderate
Germ cell mutagenicity	4	NA	4 (All no classification)	NA	Moderate
Carcinogenicity	NA	NA	NA	NA	NA
Reproductive toxicity	NA	NA	NA	NA	NA
Specific target organ toxicity single exposure	NA	NA	NA	NA	NA
Specific target organ toxicity repeated exposure (oral)	1	NA	1 (No classification)	NA	Moderate
Specific target organ toxicity repeated exposure (inhalation)	1	NA	1 (No classification)	NA	Moderate

4.1.6 Hazard classification of fullerenes

Current data suggests it is difficult to assign any hazard classification to fullerenes.

Table 4.1.4 Hazard categorization of fullerenes

HEALTH HAZARDS	HAZARD CATEGORY	EVIDENCE GRADE
Acute toxicity	No hazard categorization	Strong
Skin corrosion/irritation	No hazard categorization	Moderate
Serious eye damage/eye irritation	No hazard categorization	Moderate
Respiratory or skin sensitization	No hazard categorization	Moderate
Germ cell mutagenicity	No hazard categorization	Moderate
Carcinogenicity	No data to classify	NA
Reproductive toxicity	No data to classify	NA
Specific target organ toxicity single exposure	No data to classify	NA
Specific target organ toxicity repeated exposure	No hazard categorization (for inhalation and oral)	Moderate

Note: no data to classify: there were no data to use for classification; no hazard categorization the given data could not lead to hazard classification.

4.2 SWCNTs

4.2.1 The physicochemical properties of SWCNTs.

The SWCNTs evaluated for hazard categorization are listed in Table 4.2.1. These SWCNTs were reference materials, or alternative reference materials, for the OECD sponsorship programme for the testing of manufactured nanomaterials. Their extensive physicochemical characterization data can be found in the following published dossiers (http://www.oecd.org/chemicalsafety/nanosafety/single-walled-carbon-nanotubes-swcnts-manufacturednanomaterial.htm).

Table 4.2.1 Physicochemical properties of SWCNTs

NANO-MATERIAL	DOSSIER NO.	REF ID[a]	MANUFACTURER	CHARACTERISTICS
SWCNTs	No 50. ENV/JM/MONO (2015)13/ Part1, Part2	A	Nikkiso	Width = 43.6 nm (SD = 1.6) Length = 0.69 µm (SD = 2.1)
		B	Super growth C100	Average diameter of tubes = 8.2 nm (SD = 1.7) Average length of tubes = 0.23 µm (SD = 1.8), measured by TEM Samples in testing solution: Tube diameter: 12.0 ± 6.5 nm (mean ± standard deviation) Length: 0.51 ± 1.6 µm, measured by AFM Specific surface area: 1064 m^2/g
		C	CNI SWCNT	SWCNTs were from CNI by high-pressure catalytic CO conversion (HiPco method). SWCNTs were 1.0 ± 0.2 nm in diameter and several hundred nanometers to several micrometres long; mass mode aerodynamic diameter was 4.2 µm; diameters 1–4 nm length 0.5–1 µm; surface area: 1040 m^2/g
		D	Elicarb SWCNT	Primary particle size was 0.9–1.7 nm diameter and a fibre length < 1 µm (Thomas Swan and Co Ltd, Consett, UK)
		E	Arc method SWCNT	No information
		G	NIST SWCNT	>50% single-walled; ~40% other nanotubes; outer diameter 1.1 nm (0.7–1.2 nm), length 0.5–100 µm; manufactured by chemical vapour deposition; diameters ranged from 0.8 to 2.0 nm

NANO-MATERIAL	DOSSIER NO.	REF ID[a]	MANUFACTURER	CHARACTERISTICS
		H	Heji SWCNT	Average outside diameter of 1.1 nm, an average length of 50 µm
		I	COCC SWCNT	RNT (raw SWCNT, HiPco product of Rice); PNT (purified HiPco product of Rice); CNT (CarboLex's electric-arc product)
		J	Cheap Tubes SWCNT	No information
		K	CarboLex SWCNT	Diameters 12 nm and lengths ranging from tens of nanometers to several micrometres
		L	Rice University SWCNT	No information
		M	Dupont SWCNT	1.4 nm diameter; >1 µm length; agglomerated "ropes" of nanotubes of ~30 nm diameter; external diameters of less than 2 nm with lengths ranging from 0.5 to 40 microns
		N	Helix SWCNT	No information
		O	SWCNT AIST	Synthesized by the National Institute of Advanced Industrial Science and Technology, Japan Primary particle: maximum length, 1200 µm, diameter: 3.0 ±1.1 nm Fe, 145; Ni, 103; Cr, 34; Mn, 2; Al, 12 ppm; aggregates: length, 0.32 µm Width, 12.0 ± 6.5 nm
		P		SWCNT (4-15 µm, length; < 2 nm, diameter; 90% purity) produced by Shenzen Nanotech Port Co Ltd., China

AFM:atomic force microscopy; Al: Aluminium; CNI: CNT: CO:Cobalt Cr:Chromium; Fe:Iron; HiPco: High-Pressure CO Conversion; Mn:Manganese Ni:Nickel; ppm:parts per million; RNT:Rosette nanotubes; SD: standard deviation; TEM:transmission electron microscopy

[a] The letters in this column are used to identify the manufacturers discussed in the text.

4.2.2 Acute toxicity of SWCNTs

The acute oral and inhalation toxicity of three SWCNTs have been studied in experimental animals. An acute oral study conducted for SWCNT (A) (see Table 4.2.1) based on TG 423 showed no mortality with LD50 > 50 mg/kg/bw (Matsumoto et al., 2012). Maximum concentration could not be used due to high volume per mass. Other acute oral toxicity tests were done together with in vivo micronucleus tests based on TG 474. In these cases SWCNTs were dosed once per day for two days. SWCNT (A) and SWCNT (B) did not show any mortality up to 20 mg/kg/day and LD 50>200 mg/kg bw, respectively (Naya et al., 2011). Only one study on acute inhalation toxicity was conducted on C57BL female mice with one concentration at 5.52 ±1.37 mg/m^3 for 4 days (5 times/day). No mortality was observed. The study reported that SWCNT inhalation was more effective than aspiration in causing inflammatory response, oxidative stress, collagen deposition and fibrosis as well as mutations of K-ras gene locus in the lungs of mice (Shvedova et al., 2008). Since SWCNTs were very difficult to produce in the same large amounts as MWCNTs, it is very difficult to conduct acute toxicity tests based on the test guideline of a maximum concentration of 5 g/m^3. Therefore, no acute toxicity hazard classification was assigned for SWCNTs, with moderate to strong evidence for oral and moderate to weak evidence for inhalation.

4.2.3 Dermal and eye irritation/corrosion of SWCNTs

Studies of SWCNT (A) and SWCNT (B), based on TG 404, showed they did not cause irritation and corrosion to the skin (Ema et al., 2011). But another study using immune-competent hairless SKH-1 mice did cause dermal toxicity with induction of free radical generation, oxidative stress, and inflammation (Murray et al., 2009). Eye irritation and corrosion studies conducted for SWCNT (A) and SWCNT (B), based on TG 405, showed they did not cause eye irritation and corrosion. Hence, SWCNTs were not assigned for hazard categorization. However, caution

should be exercised for irritation because MWCNTs, which have similar material properties, did show category 2 eye irritation/corrosion with strong evidence.

4.2.4 Sensitization of SWCNTs

Two SWCNTs (A and B) were studied for sensitization based on TGs 406 and 429. They had no sensitizing effect (Ema et al., 2011) with moderate to strong evidence.

4.2.5 Specific target organ toxicity arising from repeated exposure to SWCNTs

SWCNTs have been tested subacutely based on TG 407 with compliance to GLP (Matsumoto et al., 2012). The authors found no treatment-related changes to body weight; behavioural and blood biochemical parameters were observed. NOAEL of repeated oral dose toxicity of the SWCNT was considered to be 12.5 mg/kg bw/day. It is very difficult to categorize the specific target organ toxicity.

SWCNT (A) and SWCNT (B) were tested for repeated dose toxicity through inhalation subacutely based on TG 412. SWCNT (A) exposed up to 0.4 mg/m^3 induced increased neutrophil cells in blood at 3 months after administration in the high concentration group. Neither the low concentration exposure group nor the high concentration exposure group showed an increase in the pulmonary wet weight, the infiltration of the inflammatory cell and increase of the HO-1 gene. SWCNT (B) exposed up to 0.13 mg/m^3 did not induce neutrophil inflammation in the lungs under the conditions in the present study. A no adverse effect concentration (NOAEC) of 0.13 mg/m^3 was suggested. Thus these repeated inhalation toxicity data categorize SWCNTs as specific target organ toxicity by inhalation exposure category 1 with weak evidence.

4.2.6 Genetic toxicity of SWCNTs

SWCNTs (A, B and C), were tested for in vitro genotoxicity based on TG 471. All SWCNTs were negative for bacterial reverse mutation assay, with or without metabolic activation (Naya et al., 2011; Kisin et al., 2007). Chromosomal aberration tests conducted for SWCNTs (A, B and C) were negative (Naya et al., 2011; Kisin et al., 2007). In vitro mammalian cell micronucleus tests conducted for SWCNT (E) and SWCNT (F), were positive for both SWCNTs (Cveticanin et al., 2010; Lindberg et al., 2009). In vitro comet assays conducted for SWCNTs (C, D, F, G, H and I) were positive for SWCNT (C, F, G and I) (Kisin et al., 2007; Jacobsen et al., 2008; Migliore et al., 2010; Lindberg et al., 2009; Pacurari et al., 2008; Zeni et al., 2008; Yang et al., 2009) and negative for SWCNT (H) (Jacobsen et al., 2008).

In vivo genotoxic studies for SWCNT (A and B) based on the TG 474 mammalian erythrocyte micronucleus test were negative for both SWCNTs (Naya et al., 2011). In vivo DNA damage and/or repair mouse spot tests were positive for SWCNT (C and D) (Li et al., 2007; Jacobsen et al., 2009). Interestingly SWCNT (D) was only positive for intratracheal administration, which is comparable to SWCNTs exposure route and negative for oral administration (Folkmann et al., 2009). Although SWCNT (C) was negative in in vitro standard tests, such as the bacterial reverse mutation assay and the chromosomal aberration test, it was positive for mitochondrial DNA damage (Li et al. 2007), increased K-ras mutation (Shvedova et al., 2008) and induced mitotic spindle disruption (Sargent et al., 2009). Thus SWNCTs can be classified as category 2B germ cell mutagenicity with weak evidence.

Table 4.2.2 Summary of genotoxicity of SWCNTs

SWCNT STUDIES	BACTERIAL REVERSE MUTATION ASSAY	CHROMOSOMAL ABERRATION	IN VITRO MAMMALIAN CELL MICRONUCLEUS TEST	IN VITRO SINGLE CELL GEL/ COMET ASSAY IN MAMMALIAN CELLS FOR DETECTION OF DNA DAMAGE	IN VIVO MAMMALIAN ERYTHROCYTE MICRONUCLEUS TEST	DNA DAMAGE AND/OR REPAIR, MOUSE SPOT TEST
SWCNT (A)	negative	negative	No data	No data	negative	No data
SWCNT (B)	negative	negative	No data	No data	negative	No data
SWCNT (C)	negative	negative	No data	(+)	No data	(+) mitochondrial DNA damage; increased K-ras mutation frequency; mitotic spindle disruption
SWCNT (D)	No data	No data	No data	negative	No data	negative oral (+) inhalation
SWCNT (E)	No data	No data	(+), (+)	No data	No data	No data
SWCNT (F)	No data	No data	(+)	(+)	No data	No data
SWCNT (G)	No data	No data	No data	(+), (+)	No data	No data
SWCNT (H)	No data	No data	No data	negative	No data	No data
SWCNT (I)	No data	No data	No data	(+)	No data	No data

4.2.7 Reproductive and developmental toxicity of SWCNTs

Developmental toxicity and teratogenicity studies were conducted in CD-1 mice. SWCNTs were injected intravenously at 0.01, 0.1, 0.3, 3.3 μg/mouse in the retrobulbar venous plexus of pregnant females at post-coital day 5.5. At 155 DPC, animals were sacrificed and fetus and placentas were analysed. At 30 μg/mouse-dose, SWCNTs, but not nanoCB (carbon black), were able to induce gross fetal morphologica1 abnormalities. In addition, a substantial percentage of SWCNT-exposed mice (ranging from 19% to 31%) presented swollen uteri (at least twice the diameter of a non-pregnant normal uterus) with no developed embryos – a finding never observed in control females.

A wide variety of malformations were found, but no differences in their type and severity were observed among the three groups of SWCNTs and among different concentrations within each group. In some cases the fetuses appeared morphologically normal, but significantly growth retarded; more often, fetuses with abdominal wall or head deformities or limb hypoplasia were observed. In more abnormal fetuses, a severe retardation in the development of several organs and tissues was associated with an abnormal torsion of the trunk, or the body plan was profoundly affected and the outlines of the fetus could be barely identified.

This suggested that exposure to SWCNTs may represent a potential risk for pregnant women, especially in the occupational setting, where the risk of accidental exposure may become real in light of the foreseen increased production of engineered nanomaterials (ENM) in the near future (Pietroiusti et al., 2011). Although these data suggest some form of developmental toxicity, the experiment was not conducted based on test guidelines and the exposure route was not the normal one for SWCNTs, thus it is difficult to categorize reproductive and developmental toxicity.

4.2.8 Carcinogenicity of SWCNTs

There is no data for carcinogenicity in the OECD dossiers for SWCNTs, but two reports were found in other papers (Kobayashi et al., 2011; Varga & Szendi, 2010).

The carcinogenicity of SWCNT (C) was tested in male SD rats with single intratracheal injection of the SWCNT. Eight-week old SD rats were intratracheally-instilled with AIST SWCNT (O) and sacrificed at 24 hours, 3 days, 1 week, 1 month and 3 months. In a second experiment, rats were administered with a single dose of 1 mL/kg bw of a 0 (vehicle), 0.04, 0.2, or 1.0 mg/mL solution of SWCNT in Tween 80 (doses corresponding to 0.0, 0.4, 0.2 and 1.0 mg/kg bw), and sacrificed at 3 days, 1 week, 1 month, 3 months and 6 months. No tumours were found in any group (Kobayashi et al., 2011). The experiment was too short to observe any tumours.

Groups of six F344 rats were injected with a single dose of SWCNT at 10 mg/rat intraperitoneally using the Kertai fold model, a capsular structure constructed by the ligament of minor omentum attached to the concave-shaped inner surface of the liver lobe, then observed for 12 months. As a negative control, zinc oxide was used. Mesotheliomas were not found but foreign body granulomatous lesions were observed in the Kertai fold (Varga & Szendi, 2010).

The IARC concluded that two studies with SWCNTs in rats were inconclusive and in relation to their carcinogenicity in humans categorized them as group 3 (Grosse et al., 2014; IARC 2017).

4.2.9 Other toxicological studies on SWCNTs

Other studies using intratracheal instillation, pharyngeal aspiration and intraperitoneal injection of SWCNTs were conducted in vivo experimental animals with various doses and observation periods. The results of the intratracheal instillation and pharyngeal aspiration studies showed some degree of lung damage with elevation of various biomarkers. However these studies were not conducted according to normal exposure routes and test guidelines so it was difficult to categorize the respective SWCNTs based on GHS classification.

4.2.10 Hazard classification of SWCNTs

Studies on the respiratory toxicity of SWCNTs by intratracheal instillation or pharyngeal aspiration studies indicated that repeated exposure to SWCNTs may cause lung damage and can be categorized as specific target organ toxicity repeated exposure category 1, with low evidence. The results of two studies were not consistent, so evidence was downgraded.

Table 4.2.3 Data quality of SWCNT studies

HEALTH HAZARDS	NO. OF STUDIES	NO. OF HIGH QUALITY STUDIES (HAZARD CATEGORY)	NO. OF MEDIUM QUALITY STUDIES (HAZARD CATEGORY)	NO. OF LOW QUALITY STUDIES (HAZARD CATEGORY)	EVIDENCE GRADE
Acute toxicity (oral)	3	2 (all none)	1 (none)	NA	Strong
Acute toxicity (inhalation)	1	NA	1 (none)	NA	Moderate
Skin corrosion/irritation	4	2 (all none)	2 (all none)	NA	Strong
Serious eye damage/eye irritation	2	2 (all none)	NA	NA	Strong
Respiratory or skin sensitization	3	2 (all none)	1(none)	NA	Strong
Genetic toxicity (in vitro)	17	3	14	NA	NA
Genetic toxicity (in vivo)	7	2 (all none)	5 (2 none, 3 category 2)	NA	Moderate → Weak
Carcinogenicity (OECD)	NA	NA	NA	NA	NA
Carcinogenicity (other study)	2	NA	2 (all none)	NA	Moderate
Reproductive toxicity	1	NA	1 (ambiguous)	NA	Moderate → Weak
Specific target organ toxicity single exposure	NA	NA	NA	NA	NA

HEALTH HAZARDS	NO. OF STUDIES	NO. OF HIGH QUALITY STUDIES (HAZARD CATEGORY)	NO. OF MEDIUM QUALITY STUDIES (HAZARD CATEGORY)	NO. OF LOW QUALITY STUDIES (HAZARD CATEGORY)	EVIDENCE GRADE
Specific target organ toxicity repeated exposure (oral)	1	1 (none)	NA	NA	Moderate
Specific target organ toxicity repeated exposure (inhalation)	2	NA	2 (none, category 1)	NA	Moderate → Weak

OECD: Organisation for Economic Co-operation and Development; NA: not applicable/no studies

Table 4.2.4 Hazard classification of SWCNTs

HEALTH HAZARDS	HAZARD CATEGORY	EVIDENCE GRADE
Acute toxicity	No hazard categorization (Oral) No hazard categorization (Inhalation)	Strong Moderate
Skin corrosion/irritation	No hazard categorization	Strong
Serious eye damage/eye irritation	No hazard categorization	Strong
Respiratory or skin sensitization	No hazard categorization	Strong
Germ cell mutagenicity	Category 2B: substance which causes concern owing to the possibility that it may induce heritable mutations in human germ cells	Weak
Carcinogenicity	No data to classify (# No hazard categorization based on other two studies)	NA
Reproductive toxicity	No hazard categorization (ambiguous)	Weak
Specific target organ toxicity single exposure	No data to classify	NA
Specific target organ toxicity repeated exposure	Category 1 (inhalation): substance that produced significant toxicity in humans, or based on evidence from studies in experimental animals	Weak

4.3 MWCNTs

4.3.1 Physicochemical properties of MWCNTs (multi-walled carbon nanotubes).

The MWCNTs evaluated for hazard categorization are listed in Table 4.3.1. These MWCNTs were reference materials, or alternative reference materials, for the OECD sponsorship programme for the testing of manufactured nanomaterials. Their extensive physicochemical characterization data can be found in the following published dossiers (http://www.oecd.org/chemicalsafety/nanosafety/multi-walled-carbon-nanotubes-mwcnts-manufacturednanomaterial.htm).

Table 4.3.1 Physicochemical properties of MWCNTs

NANO-MATERIAL	DOSSIER NO.	REF ID[a]	MANUFACTURER	CHARACTERISTICS
MWCNTs	No.49 ENV/JM/MONO(2015)12/Part1, part2, Part3	A	Baytube	380-902 nm (D90 = 980-1820 nm) In all cases length D90 was lower than 5 µm Mass median diameter 400 µm Specific surface area ca. 253 m^2/g
		B	Arkema Graphistrength C100	Mass median diameter 416.2 µm Agglomerate/aggregate diameter 1.5 µm (GSD 1.67) Average internal diameter: 4.8 nm Average external diameter: 11.7 nm Average length: 1097 nm Average number of walls: 10 Specific surface area 212 m^2/g
		C	Nanocyl NC 7000	Mass median diameter 85 µm Specific Surface Area ca. 230 m^2/g
		D	Nikkiso MWCNT	Diameter = 48 nm, (SD = 1.1) Length = 0.94 µm (SD = 2.3) Specific surface area ca. 69.4 m^2/g
		E	Nikkiso MWCNT B	No information
		F	Hanhwa CM-100	Length distribution was 543.3 ± 230 and 10451 ± 8421.6 nm Diameter: 10 to 15 nm Length: less than 20 µm Specific surface area ca. 224.9 m^2/g
		G	Mitsui MWCNT-7	Diameter 70-170 nm, Length 1-19 µm, >5 µm: 27.5% Specific surface area ca. 23 m^2/g
		NT50a (– agg)	Mitsui MWCNT7	Aggregated 5.29 µm, 49.95 nm. No aggregation. Same number of fibres as in the NT145 suspension
		NT50a	Mitsui MWCNT7	5.29 µm, 49.95 nm. High level of aggregation
		NT50b	Showa Denko	60 µm, 52.40 nm. High level of aggregation
		NT145	Showa Denko	4.34 µm, 143.6 nm. Low level of aggregation
		NTtngl	Showa Denko	2–20 nm diameter, Tangle

GSD: geometric standard deviation; L: litre; SD: standard deviation; OECD: Organisation for Economic Co-operation and Development; D90: D90 is the diameter at which 90% of the sample's mass is comprised of particles with a diameter less than this value.

[a] The letters in this column are used to identify the manufacturers discussed in the text.

4.3.2 Acute toxicity of MWCNTs

Acute oral, dermal and inhalation toxicity have been studied in experimental animals. Acute oral and dermal studies conducted based on TGs 420, 423 & 474 for oral, 402 for dermal and 403 for inhalation, using various MWCNTs. Many oral acute toxicity studies could not make the maximum dose of 2000 mg/kg/bw due to the large volume of MWCNTs per mass. The acute inhalation toxicity of the MWCNT (A) (see Table 4.3.1) study based on TG 403 did not show any mortality up to 241 mg/m^3 (the highest concentration that can be generated for Baytube). Therefore, no acute toxicity hazard categorization was assigned for MWCNTs, with moderate evidence for oral and strong evidence for dermal and inhalation.

4.3.3 Dermal and eye irritation/corrosion of MWCNTs

Studies of dermal irritation/corrosion caused by MWCNTs based on TG 404 and TG 431 showed they did not cause irritation and corrosion to skin. However some MWCNTs, such as MWCNT (B and E), tested in line with TG 405 were found to be irritants to eyes with strong evidence and were therefore assigned to categories 2A and 2B respectively (Ema et al., 2011).

4.3.4 Sensitization of MWCNTs

Several MWCNTs, including MWCNTs (A, B and G), were studied for sensitization based on TGs 406 and 429. The MWCNTs had no sensitizing effect.

4.3.5 Specific target organ toxicity arising from repeated exposure to MWCNTs

MWCNTs have been tested based on TG 407 and 420 (this is a fixed dose method for acute toxicity study but was modified for repeated doses) for 28 days oral toxicity and showed no toxic effect up to 0.5 mg/kg bw/day for MWCNT (C) and 50 mg/kg bw/day for MWCNT (D) (Matsumoto et al., 2012). Thus, specific target organ toxicity by orally repeated exposure to MWCNTs could not be assigned.

Six kinds of MWCNTs were tested for repeated dose toxicity through inhalation toxicity subacutely and subchronically. MWCNT [a)] exposed up to 6 mg/m^3 induced lung damage including upper respiratory tract (goblet cell hyper- and/or metaplasia, eosinophilic globules, focal turbinate remodelling) and the lower respiratory tract (inflammatory changes in the bronchioloalveolar region, increased interstitial collagen staining). Granulomatous changes and a time-dependent increase of a bronchioloalveolar hyperplasia occurred at 6 mg/m^3. The suggested NOAEL was 0.1 mg/m^3. Therefore MWCNT [a)] can be classified as category 1.

MWCNT (B) was used to expose animals up to 1.3 mg/m^3 for 5 days and then allowed to recover for 28 days. A slight increase in neutrophil count was observed at 1.30 mg/m^3. Protein levels were increased at middle and high dose after exposure and 4-week recovery and macrophage infiltration was observed at high dose after 5-day exposure and recovery. Hypertrophy of the bronchial and bronchiolar cells was observed at a high dose after exposure and recovery. Although it is difficult to categorize hazard data from a short-term inhalation study, relatively low concentrations of MWCNTs caused lung inflammation. Thus MWCNT (B) can be classed as category 1.

MWCNT (C) was used to expose animals up to 2.5 mg/m^3 for 90 days. MWCNT induced lung weight increase, pronounced multifocal granulomatous inflammation, diffuse histiocytic and neutrophilic inflammation, and with intra-alveolar lipoproteinosis even at 0.1 mg/m^3, there was still minimal granulomatous inflammation in the lung and in lung-associated lymph nodes. NOAEL would be lower than 0.1 mg/m^3. Thus MWCNT (B) can be classed as category 1.

MWCNT (D) was used on exposed animals at only 0.37 mg/m^3 for 90 days. Although fewer pulmonary inflammation responses were observed compared with intratracheal instillation, it is very difficult to make any judgement from a one-dose inhalation study.

MWCNT (F) was tested for short-term and subacute inhalation toxicity. The 5-day short-term inhalation exposure to MWCNT increased hydrogen peroxide in the bronchoalveolar lavage (BAL) fluid even after 1 month post-exposure. In the 28-day exposure study with 90-day recovery, the inflammatory cytokine levels in the BAL fluid did not show any statistically significant difference. The H_2O_2 levels in the BAL were significantly higher in the middle- (0 days post-exposure) and high- (0 days and 28 days post-exposure) dose groups. The short-length MWCNTs deposited in the lung cells were persistent at 90 days post-exposure. Although there were some increases in H_2O_2 in the BAL in both studies, distinct increases in other inflammatory biomarkers and histopathological legions were not observed. Thus, it is difficult to categorize the MWCNTs.

MWCNT (F) was tested for short-term and subacute inhalation toxicity. Subacute inhalation at 10 mg/m^3 exposure to MWCNTs induced dose-dependent pulmonary inflammation and damage with rapid development of pulmonary fibrosis, and also demonstrated that MWCNTs can reach the pleura afterwards (Porter et al., 2012). Subchronic exposure to MWCNTs increased lung weights 1.2-fold with 1 mg/m^3 and 1.3-fold with 5 mg/m^3 in both sexes compared to the controls. In the bronchoalveolar lavage fluid analyses, inflammatory parameters were increased concentration-dependently in both sexes from 0.2 mg/m^3.

Granulomatous changes in the lung were induced at 1 and 5 mg/m^3 in females and even at 0.2 mg/m^3 in males. Focal fibrosis of the alveolar wall was observed in both sexes at 1 mg/m^3 or higher. Inflammatory infiltration in the visceral pleural and subpleural areas was induced only at 5 mg/m^3. The low-observed-adverse-effect level (LOAEL) for respiratory tract toxicity in the present inhalation exposure study of rats was 0.2 mg/m^3. Therefore NOAEL would be much lower than the 0.2 mg/m^3 LOAEL.

Thus several studies on repeated inhalation exposure indicated that MWCNT (F) can be classed as category 1 with high evidence.

4.3.6 Genotoxicity of MWCNTs

The genotoxicity of MWCNTs was extensively studied in in vitro bacterial reverse mutation assay (based on TG 471) (Wirnitzer et al., 2009; Ema et al., 2012; Kim et al., 2011) and chromosomal aberration assay (based on TG 473) (Wirnitzer et al., (2009); Ema et al., 2012; Asakura et al., 2010; Kim et al., 2011), and in–vitro mammalian cell gene mutation tests based on TG 476 (Asakura et al., 2010), or non-guideline based test. MWCNTs were also tested in vivo micronucleus assays (TG 474) (Kim et al., 2011; Ema et al., 2012) and comet assay (TG 489) (Table 4.3.2) (Kim et al., 2012; 2014). Most studies were conducted in compliance with GLP and MWCNTs were exposed intraperitoneally (Kim et al., 2011), orally (Ema et al., 2012) or by inhalation (Kim et al., 2012; 2014).

All MWCNTs tested by in vitro bacterial reverse mutation assay were negative for genotoxicity. MWCNT (A, B and F) evaluated by in vitro chromosomal aberration tests were negative and MWCNT (B and F) evaluated by in vitro mammalian cell gene mutation test were negative. MWCNT (D and G) evaluated by chromosomal aberration were positive and MWCNT (G) evaluated by in vitro mammalian cell gene mutation test was negative but positive with in vitro mammalian cell micronucleus test. MWCNT (D) exposed orally (Ema et al., 2012), MWCNT (F) exposed intraperitoneally (Kim et al. 2011) and MWCNT (G) administered orally (Ema et al., 2012) evaluated by in vivo mammalian erythrocyte micronucleus test, were negative but MWCNT (F) exposed by inhalation acutely or subacutely was positive to lung cells with comet assay (Kim et al. 2012; 2014).

Therefore MWCNTs with agglomerated/aggregated form like Baytube and Graphistrength were negative for genotoxicity in vitro, but MWCNTs having rigid structure such as Nikkiso and MWCNT-7 (previously called Mitsui MWCNT) were positive for in vitro genotoxicity test. In vivo micronucleus tests conducted by oral and intraperitoneal administration of MWCNTs were negative, but comet assays to the lung cells that are actually exposed with inhalation were positive. Therefore most CNTs if exposed with a dispersed CNT structure can be genotoxic. Taken together with the limitation of in vivo dosing data, MWCNTs could be classed as category 2 mutagens with strong evidence.

Table 4.3.2 Summary of genotoxicity of MWCNTs

NANO-MATERIAL	BACTERIAL REVERSE MUTATION ASSAY	CHROMO-SOMAL ABERRATION	IN VITRO MAMMALIAN CELL GENE MUTATION TEST	IN VITRO MAMMALIAN CELL MICRONUCLEUS TEST	MAMMALIAN ERYTHROCYTE MICRO-NUCLEUS TEST	COMET ASSAY
MWCNT (A)	negative	negative	negative	No data	No data	No data
MWCNT (B)	negative	negative	negative	No data	No data	No data
MWCNT (C)	negative	No data	No data	No data	No data	No data
MWCNT (D)	negative	(+)	No data	No data	negative oral	No data
MWCNT (E)	No data		No data	No data	No data	No data
MWCNT (F)	negative	negative	No data	negative	negative IP	(+) inhalation
MWCNT (G)	negative	(+)/(+)	negative	(+)	negative oral	No data

4.3.7 Reproductive and developmental toxicity of MWCNTs

Reproductive and developmental toxicity was studied in Institute of Cancer Research (ICR) mice and Sprague Dawley rats based on TG 414. The study conducted by Fujitani et al., (2012) with two different routes of exposure, intraperitoneal and intratracheal instillation that are not usual routes of exposure recommended by TG 414 in which oral route is recommended, showed that the number of litters having fetuses with external malformations, and those litters having fetuses with skeletal malformations, were both increased in proportion to the doses of MWCNT. Although the authors suggested that MWCNT has a potency of teratogenicity, at least under the present experimental conditions the doses used in this study, in terms of large volumes per mass, were so extreme that this may have caused the teratogenicity.

Other studies conducted according to TG 414 (prenatal development toxicity study) showed that the repeated oral dose of MWCNTs during pregnancy induced minimal maternal toxicity and no embryo-fetal developmental toxicity at 1000 mg/kg bw/day in rats. The NOAEL of MWCNTs is considered to be 200 mg/kg bw/day for dams (pregnant female rat) and 1000 mg/kg bw/day for embryo-fetal development. Therefore MWCNTs may not be categorized to reproductive toxicity.

4.3.8 Carcinogenicity of MWCNTs

The carcinogenicity of MWCNT (C) was tested in male Wistar rats with a single injection of MWCNT, with defect and without defect, at two doses. The incidence of tumours other than mesothelioma was not significantly increased across the groups (Muller et al., 2009). MWCNT (G) was extensively studied using intraperitoneal, intrascrotal and inhalation routes of exposure.

Takagi et al. (2008 and 2012) studied the carcinogenicity of MWCNT (G) (MWCNT-7) initially with relatively high doses and then low doses in p53 (+/-) heterozygous mice. After the high-dose intraperitoneal injection, the MWCNTs induced mesothelioma in the p53(+/-) mice. Later lower-dose intraperitoneal administration also induced mesotheliomas with incidences of 19/20, 17/20 and 5/20, respectively. This suggested that the severity of peritoneal adhesion and granuloma formation were dose-dependent and minimal in the lowest dose group. However, the time of tumour onset was apparently independent of the dose.

Another study that administered MWCNTs to Fischer 344 rats intrascrotally also showed similar results with mesothelioma induction (Sakamoto et al., 2009). Nagai et al., (2011) studied carcinogenicity using aggregated and non-aggregated, thin and thick, and tangle MWCNTs. They found that thin MWCNTs (diameter ~ 50 nm) with high crystallinity showed mesothelial cell membrane piercing and cytotoxicity in vitro and subsequent inflammogenicity and mesotheliomagenicity in vivo. In contrast, thick (diameter ~ 150 nm) or tangled (diameter ~2–20 nm) MWCNTs were less toxic, inflammogenic and carcinogenic. Thin and thick MWCNTs similarly affected macrophages. The tangled MWCNTs hardly caused any mesothelioma.

Pretreatment of methylcholanthrene (MCA) to male B6C3F1 mice prior to inhalation exposure to MWCNTg) for 15 days at 5 mg/m^3 induced lung bronchioloalveolar adenomas and lung adenocarcinomas. Thus this result suggested that MWCNT exposures promote the growth and neoplastic progression of initiated lung cells in B6C3F1 mice.

Rittinghausen et al. (2014) administered tailor-made MWCNTs manufactured from various carbon sources such as benzene, cyclohexane and acetonitrile with a length longer than 8 μm and diameter larger than 37 nm, which were injected into Wistar rats intraperitoneally. All tested MWCNT types caused mesotheliomas with the highest frequencies and earliest appearances after treatment in the rather straight MWCNT types A and B. In the MWCNT C groups, first appearances of morbid mesothelioma-bearing rats were only slightly later. Later during the two-year study, they found mesotheliomas also in rats treated with MWCNT D, which are the most curved type of nanotubes, indicating shape may not be a critical factor in the induction of mesothelioma.

In 2014, the IARC reviewed the carcinogenicity of CNTs. The IARC working group concluded that there was sufficient evidence for MWCNT-7, limited evidence for the other two types of MWCNTs with dimensions similar to MWCNT-7, and inadequate evidence for SWCNTs. Mechanistic and other data in rodents provided evidence of translocation of three types of MWCNTs (including MWCNT-7) to the pleura. Additionally, inhalation of some MWCNTs or SWCNTs induced acute or persistent pulmonary inflammation, granuloma formation, fibrosis and bronchiolar or bronchioloalveolar hyperplasia in rodents. Studies in rodents and in cultured human lungs or mesothelial cells showed that MWCNTs, SWCNTs, or both, induce genetic lesions such as DNA strand breaks, oxidized DNA bases, mutations, micronucleus formation and chromosomal aberrations. SWCNTs and MWCNTs also perturb the cellular mitotic apparatus, including microtubules and centrosomes, in human lung epithelial cells.

As a whole, the IARC working group acknowledged that the above mechanisms are all relevant to humans. However, a majority did not consider the mechanistic evidence for carcinogenicity – especially concerning chronic endpoints – to be strong for any specific CNT. Furthermore, the lack of coherent evidence across the various distinct CNTs precluded generalization to other types of CNTs. Thus, MWCNT-7 was classified as possibly carcinogenic to humans (group 2B); and SWCNTs and MWCNTs excluding MWCNT-7 were categorized as not classifiable regarding their carcinogenicity to humans (group 3) (Grosse et al., 2014; IARC 2017).

4.3.9 Other toxicity studies of MWCNTs

Other studies using intratracheal instillation, pharyngeal aspiration and intraperitoneal injection of MWCNTs were conducted in in vivo experimental animals with various doses and observation periods. Results of these intratracheal instillation and pharyngeal aspiration studies showed some degree of lung damage with elevation of various biomarkers. However these studies were not conducted according to normal exposure routes and test guidelines so it was difficult to categorize respective MWCNTs based on GHS categorization. Specific target organ toxicity (single exposure) and aspiration hazard for MWCNTs were not studied and no data are available.

4.3.10 Hazard categorization of MWCNTs

Specific target organ toxicity (single exposure) and aspiration hazard for MWCNTs were not studied and no data are available. However, specific target organ toxicity by single exposure and aspiration hazard were not likely to be caused by MWCNT exposure based on acute, subacute and subchronic toxicity studies. MWCNTs were classified as category 2A serious eye damage/ irritation with moderate to high evidence; category 2B germ cell mutagen with moderate to strong evidence, and category 1 specific target organ (respiratory system) toxicity repeated exposure with moderate evidence. MWCNT-7 was classed as category 2 carcinogen or as IARC group 2B carcinogen. Other MWCNTs were classified as group 3 by the IARC.

Table 4.3.3 Data quality of MWCNT studies

HEALTH HAZARDS	NO. OF STUDIES	NO. OF HIGH QUALITY (HAZARD CATEGORY)	NO. OF MEDIUM QUALITY (HAZARD CATEGORY)	NO. OF LOW QUALITY (HAZARD CATEGORY)	EVIDENCE GRADE
Acute toxicity (oral)	6	2 (all none)	3 (all none)	1 (none)	Strong
Acute toxicity (dermal)	3	2 (all none)	NA	1 (none)	Strong
Acute toxicity (inhalation)	2	1 (none)	1 (none)	NA	Strong
Skin corrosion/irritation	6	1 (none)	2 (all none)	3 (all none)	Strong
Serious eye damage/eye irritation	5	2 (all category 2)	2 (all none)	1 (none)	Strong
Respiratory or skin sensitization	3	1	1	1	Strong → Moderate
Genetic toxicity (in vitro)	16	5	9	2	NA
Genetic toxicity (in vivo)	5	1 (category 2)	4 (category1, 3 none)	NA	Strong
Carcinogenicity	4 *3 (other studies)	NA	4 (3 category 2, 1 No classification) * other studies (2 category 2, 1 No classification none)	NA	Moderate
Reproductive toxicity	2	NA	2 (1 none, 1 ambiguous)	NA	Moderate
Specific target organ toxicity single exposure	NA	NA	NA	NA	NA
Specific target organ toxicity repeated exposure (oral)	1	1 (none)	NA	NA	Strong
Specific target organ toxicity repeated exposure (inhalation)	8	2 (category 1, none)	6 (category 1, category 1, category 1, none, none, none)	NA	Moderate

Table 4.3.4 Hazard categorization of MWCNTs

HAZARD CATEGORY	MWCNTS	EVIDENCE
Acute toxicity	No hazard categorization	Strong
Skin corrosion/irritation	No hazard categorization	Strong
Serious eye damage/eye irritation	Category 2: induce reversible eye irritation	Strong
Respiratory or skin sensitization	No hazard categorization	Moderate
Germ cell mutagenicity	Category 2: substances that cause concern owing to the possibility that they may induce heritable mutations in the germ cells of humans	Strong
Carcinogenicity	Category 2: suspected human carcinogen for rigid MWCNT-7, and other MWCNTS are not classifiable.	Moderate
Reproductive toxicity	No hazard categorization	Moderate
Specific target organ toxicity single exposure	No data to classify	NA
Specific target organ toxicity repeated exposure	Category 1 (inhalation): substance that produced significant toxicity in humans, or that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to produce significant toxicity in humans following repeated exposure No hazard (Oral)	Moderate Strong

4.4 Silver nanoparticles

4.4.1 Physicochemical properties of silver nanoparticles

The silver nanoparticles (AgNP) evaluated for hazard categorization are listed in Table 4.4.1. These particles were reference material, or alternative reference materials, for the OECD sponsorship programme for the testing of manufactured nanomaterials. Their extensive physicochemical characterization data can be found in the following published dossiers (http://www.oecd.org/chemicalsafety/nanosafety/silver-nanoparticles-manufactured-nanomaterial.htm).

Table 4.4.1 Physicochemical properties of silver nanoparticles

NANO-MATERIAL	DOSSIERS	REF ID[a]	MANUFACTURER	CHARACTERISTICS
AgNP	No 53. ENV/JM/MONO(2015)16/Part1, Part2, Part3, Part4, Part5, Part6, Part7	A	ABC Nano Nanotech	Average diameter 10 nm, citrated coated
		B	Namatech Co, Ltd	Average diameter 58–60, dry powder
		C	Daedeok Science	Silver nanoparticle generated by evaporation/condensation from silver wire by the method described in ISO 10801

AgNP: silver nanoparticles; ISO: International Organization for Standardization.

[a] The letters in this column are used to identify the manufacturers discussed in the text.

4.4.2 Acute toxicity of silver nanoparticles

Acute oral, dermal and inhalation toxicity have been studied in experimental animals. Acute oral and dermal studies based on TG 423 and TG 401, and TG 402 using 10 nm AgNPs, did not show any mortality up to 2000 mg/kg/bw (Kim et al., 2013). An acute inhalation toxicity study based on TG 403 (Sung et al., 2011) did not show any mortality up to 750 μg/m^3 (the highest concentration that can be generated). Therefore, no acute toxicity hazard categorization was assigned for silver nanoparticles, with strong evidence.

4.4.3 Dermal and eye irritation/corrosion of silver nanoparticles

Studies of dermal and eye irritation/corrosion caused by silver nanoparticles and based on TG 404 and TG 405 respectively, showed they did not cause any eye irritation and corrosion.

4.4.4 Sensitization of silver nanoparticles.

Skin sensitization studies conducted based on TG 406 indicated that silver nanoparticles are a weak sensitizer and can be assigned to category 1B skin sensitizer, with moderate evidence.

4.4.5 Specific target organ toxicity arising from repeated exposure to silver nanoparticles

Silver nanoparticles that were tested based on TG 407 and TG 408 resulted in a wide range of dose responses. Doses of 10 nm AgNPs did not produce significant effects in subacute and subchronic studies, showing NOAEL 250 mg/kg/bw (Hong et al., 2013). On the contrary, 58–60 nm AgNPs GLP-compliant subacute and subchronic studies showed consistent hepatic toxicity, with increases in serum alkaline phosphatase and cholesterol accompanying bile duct hyperplasia (Kim et al., 2008; 2010).

Silver nanoparticles can be a powerful intestinal secretagogue and they induce an abnormal mucin composition in the intestinal mucosa (Jeong et al., 2010). NOAELs for 28- and 90-day studies at 30 mg/mg/bw were suggested. Therefore AgNPs can be assigned to category 2. The liver and GI tract are the affected target organs.

Inhalation toxicity of silver nanoparticles was extensively studied subacutely and subchronically. Although no significant effects were observed in the subacute inhalation study based on TG 412 (Ji et al., 2007), a subchronic silver nanoparticles exposure study conducted based on TG 413 (Sung et al., 2009) in compliance with GLP, indicated that exposure to silver nanoparticles induced bile duct hyperplasia dose-dependently in both male and female rats and also induced mixed inflammatory infiltrate, chronic alveolar inflammation and small granulomatous lesions in lungs, with decreases in the lung function parameters such as tidal volume and minute volume. The target organs for silver nanoparticles were liver and lungs and a NOAEL of 133 μg/m^3.

Another subchronic study exposed rats to silver nanoparticles for 12-weeks and studied clearance of silver from organs and recovery of lung function parameters (Song et al., 2013). Male rats showed consistent lung function (tidal volume) decrease during exposure and recover period. In contrast, the female rats did not show a consistent lung function decrease either during the exposure period or following the exposure cessation. The histopathology showed a gradual recovery from the lung inflammation in the female rats, whereas the male rats in the high-dose group exhibited persistent inflammation throughout the 12-week recovery period. A NOAEL of 117 μg/m^3 was suggested in this study (Song et al., 2013).

Gender differences in the accumulation of silver in organs, including the kidneys and adrenals, have been reported in several oral and inhalation-repeated dose studies after repeated exposure to silver nanoparticles (Kim et al., 2009). Female kidneys and adrenals accumulated two to three times more silver than those of males (Kim et al., 2008 and 2009; Sung et al., 2009; Song et al., 2013). Therefore silver nanoparticles can be assigned to specific target organ toxicity repeated exposure category 1 for inhalation exposure with strong evidence.

4.4.6 Genotoxicity of silver nanoparticles

The genotoxicity of silver nanoparticles was studied in in vitro bacterial reverse mutation assay (based on TG 471) (Kim et al., 2013) and chromosomal assay (TG 473) (Kim et al., 2013) and in vivo micronucleus assays (TG 474) (Kim et al., 2008) and comet assay (489) (Table 4.4.2 (Cho et al., 2013). Most studies were conducted in compliance with GLP and silver nanoparticles were exposed both orally (Kim et al., 2008) and by inhalation (Kim et al., 2011b; Cho et al., 2013) in in vivo genotoxicity studies. All in vitro and in vivo genotoxicity studies indicated that silver nanoparticles were negative for genotoxicity; one 12-week inhalation exposure study showed a positive result at high concentrations that workers would not normally be exposed to these levels (Cho et al., 2013). Therefore, silver nanoparticles were categorized as no genotoxicity hazard with strong evidence.

Table 4.4.2 Data quality of silver nanoparticle genotoxicity studies

HAZARD CATEGORY	NO. OF STUDIES	NO. OF HIGH QUALITY	NO. OF MEDIUM QUALITY	NO. OF LOW QUALITY	EVIDENCE GRADE
Genetic toxicity (in vitro)	2	2	NA	NA	Strong
Genetic toxicity (in vivo)	3	2	1 (±)	NA	Strong

4.4.7 Reproductive and developmental toxicity of silver nanoparticles

Reproductive and developmental toxicity were studied in Sprague-Dawley rats based on TG 422 with GLP compliance. No reproductive toxicity to parental animals as well as offspring was found with strong evidence.

4.4.8 Hazard categorization of silver nanoparticles

Silver nanoparticles can be categorized as a skin sensitizer with moderate evidence. For specific target organ toxicity repeated exposure, silver nanoparticle can be categorized as category 2 for oral exposure with strong evidence and specific target organ (liver and respiratory system) toxicity repeated exposure, and category 1 for inhalation exposure, with strong evidence.

Table 4.4.3 Data quality of silver nanoparticle studies

HEALTH HAZARDS	NO. OF STUDIES	NO OF HIGH QUALITY (HAZARD CATEGORY)	NO. OF MEDIUM QUALITY (HAZARD CATEGORY)	NO. OF LOW QUALITY (HAZARD CATEGORY)	EVIDENCE GRADE
Acute toxicity (oral)	3	1 (none)	NA	2 (all none)	Strong
Acute toxicity (dermal)	1	1 (none)	NA	NA	Strong
Acute toxicity (inhalation)	1	1 (none)	NA	NA	Strong
Skin corrosion/irritation	3	2 (all none)	1 (none)	NA	Strong
Serious eye damage/eye irritation	3	1 (none)	1(none)	1 (none)	Strong
Respiratory or skin sensitization	3	1 (category 1)	NA	2 (ambiguous)	Moderate
Genetic toxicity (in vitro)	2	2 (2 none)	NA	NA	Strong
Genetic toxicity (in vivo)	3	2	1 (±)	NA	Strong
Carcinogenicity	NA	NA	NA	NA	NA
Reproductive toxicity	2	2	NA	NA	Strong
Specific target organ toxicity single exposure	NA	NA	NA	NA	NA
Specific target organ toxicity repeated exposure (oral)	6	4 (1 category 2, 3 none)	2 (all none)	NA	Strong
Specific target organ toxicity repeated exposure (inhalation)	4	3 (category 1, 2 none)	1 (category1)	NA	Strong

Table 4.4.4 Hazard categorization of silver nanoparticles

HEALTH HAZARDS	HAZARD CATEGORY	EVIDENCE GRADE
Acute toxicity	No hazard categorization	Strong
Skin corrosion/irritation	No hazard categorization	Strong
Serious eye damage/eye irritation	No hazard categorization	Strong
Respiratory or skin sensitization	Skin sensitizer category 1B	Strong
Germ cell mutagenicity	No hazard categorization	Strong
Carcinogenicity	No data to classify	—
Reproductive toxicity	No hazard categorization	Strong
Specific target organ toxicity single exposure	No data to classify	NA
Specific target organ toxicity repeated exposure	Category 1 (inhalation): Substance that produced significant toxicity in humans, or that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to produce significant toxicity in human following repeated exposure	Strong
	Category 2 (oral): Substance that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure	Strong

4.5 Gold nanoparticles

Gold nanoparticles evaluated for hazard categorization are listed in Table 4.5.1. These particles were reference material, or alternative reference materials, for the sponsorship programme for the testing of manufactured nanomaterials. Their extensive physicochemical characterization data can be found in the following published dossiers (http://www.oecd.org/chemicalsafety/nanosafety/gold-nanoparticles-manufacturednanomaterial.htm).

4.5.1 Physicochemical properties of gold nanoparticles

Table 4.5.1 Physicochemical properties of gold nanoparticles

NANO-MATERIAL	DOSSIER NO.	REF ID[a]	MANUFACTURER	CHARACTERISTICS
AuNP	No 44. ENV/JM/MONO(2015)7	A	Mintek, South Africa	Average diameter 14 ± 2 nm, citrated coated
		B	Daedeok Science, Republic of Korea	Gold nanoparticles generated by evaporation/condensation from gold wire by the method described by ISO 10801
		C	Mintek, South Africa	Average diameter 14 ± 2 nm, citrated coated

AuNPs: gold nanoparticles; ISO: International Organization for Standardization.
[a] The letters in this column are used to identify the manufacturers discussed in the text.

4.5.2 Acute toxicity of gold nanoparticles

No study results were available.

4.5.3 Dermal and eye irritation/corrosion of gold nanoparticles

No study results were available.

4.5.4 Sensitization of gold nanoparticles

No study results were available.

4.5.5 Specific target organ toxicity – repeated exposure to gold nanoparticles

Two studies on gold nanoparticle inhalation and one on intravenous injection AuNP (A) were available. Rats were exposed to gold nanoparticles of 13 nm and 105 nm by inhalation over 5 days, at concentrations of 12.8 μg/m^3 for 13 nm and 13.7 μg/m^3 for 105nm. The rats were allowed to recover for 28 days to evaluate clearance from their lungs. The results showed size-dependent clearance with faster clearance of the 14 nm particles compared to those of 95 nm. Gold nanoparticles showed biopersistence in the lung tissue; old nanoparticles did not translocate extrapulmonary to other tissues such as the kidney, brain and testis. Small numbers of gold nanoparticles translocated from the lung to the liver and spleen (Han et al., 2015).

A subchronic 90-day inhalation toxicity study on gold nanoparticles (4-5 nm), based on TG 413 in compliance with GLP, was conducted in male and female Sprague-Dawley rats with concentrations of 1.85 × 106 particles/cm^3 (20.02 μg/m^3); 2.36 × 105 particles/cm^3 (0.38 μg/m^3) and 2.36 × 104 particles/cm^3 (0.04 μg/m^3). Among the pulmonary function test parameters, there were significant changes in tidal volume and minute volume during the 90 days of gold nanoparticle exposure ($p < 0.01–0.05$). Dose-dependent tidal volume decreases in male rats led to minute volume decreases in the high-dose animals. A tendency towards a dose-dependent decrease in the tidal volume appeared to be present in female rats, but was not statistically significant. No significant differences were found between the control and any treated animals for blood clotting time measured as activated partial thromboplastin time (APPT) or prothrombin

time (PT). Tissue distribution of gold nanoparticles to the lungs, kidneys, liver, blood, brain and olfactory nerve was prominent and concentration dependent. The NOAEL of gold nanoparticles was considered to be 2.36 × 105 particles/cm^3 (0.38 μg/m^3) in rats (Sung et al., 2011). Therefore, gold nanoparticles can be assigned to category 1 with strong evidence.

Gold nanoparticles (14 nm) were injected into the tail vein of SD rats for 28 days and the rats were allowed to clear the gold nanoparticles in their tissue for 1, 2 and 4 months. No mortality or clinical signs were observed. No significant difference in body weight, food and water consumption was observed in any of the dose groups. No significant difference was observed in haematology and clinical chemistry in any of the dose groups. No significant gross effects were observed in any of the dose groups. No significant difference was observed in organ weight in any of the dose groups. No significant difference among dose group was observed for histopathology. Under the test conditions, the NOAEL of 14 nm gold nanoparticles was considered to be 100 μg/kg bw/day in male rats.

4.5.6 Genotoxicity of gold nanoparticles

Genotoxicity tests based on the TG 471 bacterial reverse mutation test for AuNP (C) were negative in the absence of metabolic activation. Chromosomal aberration tests based on TG 473 using Chinese hamster ovary (CHO) cells were positive in the absence of metabolic activation but there was no concentration-related increase in the observed aberrations. The experiment needs to be repeated to determine the reproducibility of aberrations.

Table 4.5.2 Data quality of genotoxicity of gold nanoparticles

HAZARD CATEGORY	NO. OF STUDIES	NO. OF HIGH QUALITY	NO. OF MEDIUM QUALITY	NO. OF LOW QUALITY	EVIDENCE GRADE
Genetic toxicity (in vitro)	2	NA	2 (2-)	NA	weak

4.5.7 Hazard categorization of gold nanoparticles

Gold nanoparticles can be assigned to specific target organ (respiratory system) toxicity repeated exposure category 1 for inhalation exposure, with strong evidence.

Table 4.5.3 Data quality of Specific target organ toxicity repeated exposure of gold nanoparticles

HAZARD CATEGORY	NO OF STUDIES	NO OF HIGH QUALITY (HAZARD CATEGORY)	NO OF MEDIUM QUALITY (HAZARD CATEGORY)	NO OF LOW QUALITY (HAZARD CATEGORY)	EVIDENCE GRADE
Specific target organ toxicity repeated exposure (inhalation)	2	1 (category 1)	1 (none)	NA	Strong
Specific target organ toxicity repeated exposure (injection)	1	NA	1 (none)	NA	Moderate

Table 4.5.4 Hazard categorization of gold nanoparticles

HEALTH HAZARDS	HAZARD CATEGORY	EVIDENCE GRADE
Acute toxicity	No data to classify	NA
Skin corrosion/irritation	No data to classify	NA
Serious eye damage/eye irritation	No data to classify	NA
Respiratory or skin sensitization	No data to classify	NA
Germ cell mutagenicity	No data to classify	NA
Carcinogenicity	No data to classify	NA
Reproductive toxicity	No data to classify	NA
Specific target organ toxicity single exposure	No data to classify	NA
Specific target organ toxicity repeated exposure	Category 1 (inhalation): substance that produced significant toxicity in humans, or that was expected to do so on the basis of evidence from studies in experimental animals	Strong

4.6 Silicon dioxide

4.6.1 Physical and chemical properties of silicon dioxide

Silicon dioxides that were evaluated for hazard categorization are listed in Table 4.6.1. These silicon dioxides were reference material, or alternative reference materials, for the sponsorship programme for the testing of manufactured nanomaterials. Their extensive physicochemical characterization data can be found in the following published dossiers (http://www.oecd.org/chemicalsafety/nanosafety/silicon-dioxide-manufactured-nanomaterial.htm)

Table 4.6.1 Physicochemical properties of silicon dioxide (SiO_2)

NANO-MATERIAL	DOSSIER NO.	MANUFACTURER	CHARACTERISTICS
SiO_2 (A)[a] (NW-200 silicon dioxide)	No 51. ENV/JM/MONO(2015)14/ Part1, Part2, Part3, Part4, Part5, Part6	No information	Fluffy powder, density 182 g/l, fully amorphous Primary particle size by TEM 10-15 nm Agglomerates' particle size mean >480 um Surface BET 220 m^2/g Zeta potential 45 mV around pH7
SiO_2 (B) (NW-201 silicon dioxide)		No information	Fluffy powder, density 304 g/l, fully amorphous Primary particle size by TEM 17-19 nm Agglomerates' particle size mean >238 µm Surface BET 160 m^2/g Zeta potential 40 mV around pH7
SiO_2 (C) (NW-202 silicon dioxide)		No information	Powder, density 45 g/l, fully amorphous Primary particle size by TEM 15-20 nm Agglomerates particle size mean 175.9 µm Surface BET 204 m^2/g Zeta potential 40 mV around pH7
SiO_2 (D) (NW-203 silicon dioxide)		No information	Powder, density 44 g/l, fully amorphous Primary particle size by TEM 13-16 nm Agglomerates' particle size mean >694 µm Surface BET 203 m^2/g Zeta potential 35 mV around pH7
SiO_2 (E) (NW-204 silicon dioxide)		No information	Fluffy powder, polydispersable, fully amorphous Surface BET 140 m^2/g

BET: surface to predict bioavailability; TEM: transmission electron microscopy

[a] The letters in ( ) are used to identify the nanomaterials discussed in the text.

4.6.2 Acute toxicity of silicon dioxide

The acute oral and inhalation toxicity of silicon dioxide has been studied in experimental animals (17 cases). All acute oral studies based on TG 401 showed no mortality at maximum experiment concentration. Acute inhalation toxicity studies based on TG403 showed no mortality at test concentration levels and therefore no acute toxicity hazard was assigned to silicon dioxide in both cases.

4.6.3 Dermal and eye irritation/corrosion of silicon dioxide

Dermal irritation/corrosion studies based on TG 404 or the United States Environmental Protection Agency method of five types of silicon dioxide, did not cause irritation or corrosion to skin. Eye irritation and corrosion studies based on TG 405 or the Draize test did not cause eye irritation and corrosion. Thus, silicon dioxide is unlikely to cause dermal and eye irritation/corrosion, and is not assigned for hazard categorization.

4.6.4 Specific target organ toxicity arising from repeated exposure to silicon dioxide

Silicon dioxide (A) has been tested subacutely based on TG 407 in compliance with GLP. No significant adverse effects were observed. The NOAEL of repeated oral dose toxicity of the silicon dioxide was considered to be 1000 mg/kg bw/day. It is very difficult to categorize the specific target organ toxicity. Silicon dioxides (A, B and E) were tested for repeated dose toxicity through inhalation subacutely based on TG 408. All silicon dioxides were tested up to 4500 mg/kg bw/day over a 13-week period. No clinical symptoms or other findings including haematological, blood-chemical and urinary parameters were observed.

Silicon dioxide (A) has been tested subacutely based on TG 413 in compliance with GLP. The test level of 35 mg/m^3 induced generally mild changes, but subjects quickly recovered during the exposure period. NOAEC were not identified and therefore were assigned as none. Silicon dioxide (A) has been tested subacutely based on TG 412 in compliance with GLP. The high exposure concentration (25.2 mg/m^3) induced substance-related effects which reflect an inflammatory response in the lung tissue. These effects tended to disappear during the recovery phase, although apparently not completely, but show clear signs of reversibility. The effects of the mild exposure concentration (5.39 mg/m^3) were confined to a very slight increase in the relative neutrophil count with a concomitant decrease in the relative macrophage count on the day after exposure, but this was only statistically significant in males. NOAEC could be defined as 5.39 mg/m^3 and assigned as category 2.

Silicon dioxide (B) has been tested subacutely based on TG 413 in compliance with GLP. The test level of 35 mg/m^3 induced generally mild changes, but subjects quickly recovered during the exposure period. NOAEC were not identified and were therefore assigned as none. Silicon dioxide (B) has been tested subacutely based on TG 412 in compliance with GLP. The high exposure concentration (25.2 mg/m^3) induced substance-related effects which reflect an inflammatory response in the lung tissue with morphological tissue reaction. These tended to disappear during the recovery phase, although apparently not completely, but show clear signs of reversibility. The effects of the mild exposure concentration (5.39 mg/m^3) were confined to a very slight increase in the relative neutrophil count with a concomitant decrease in the relative macrophage count on the day after exposure, but this was only statistically significant in males. NOAEC could be defined as 5.39 mg/m^3 and assigned as category 2.

Silicon dioxide (C) has been tested subacutely based on TG 413 in compliance with GLP. Inhaled silica at low concentrations provokes an inflammatory response in the respiratory tract of rats, particularly in the lungs. All silica was completely cleared from the lungs. An increased respiration rate was noted as a clinical sign. NOAEC could be defined as 1.3 mg/ m^3 and assigned as category2. Another study tested silicon dioxide (C) subacutely based on TG 412 in compliance with GLP. The mild and high exposure concentrations (5 mg/m^3 and 25.2 mg/

m^3) induced substance- and dose-related effects, which reflect an inflammatory response in the lung tissue. These tended to disappear during the recovery phase. The lymph nodes were also affected. NOAEC could be defined as 1.39 mg/m3 and assigned as category 2.

Silicon dioxide (D) has been tested subacutely based on TG 413 in compliance with GLP. Inhaled silica at low concentrations provokes an inflammatory response in the respiratory tract of rats, particularly in the lungs. All silica was completely cleared from the lungs. An increased respiration rate was noted as a clinical sign. NOAEC could be defined as 1.3 mg/ m^3 and assigned as category 2. Another study tested silicon dioxide (D) subacutely based on TG 412 with compliance to GLP. The mild and high exposure concentrations (5 mg/m3 and 25.2 mg/ m^3) induced substance- and dose-related effects, which reflect an inflammatory response in the lung tissue. These tended to disappear during the recovery phase. The lymph nodes were also affected. NOAEC could be defined as 1.39 mg/m^3 and assigned as category 2.

Silicon dioxide (E) has been tested subacutely based on TG 413 in compliance with GLP. No clinical symptoms or other findings were observed. No NOAEC were identified and were therefore assigned as none. Another study tested silicon dioxide (E) subacutely based on TG 412 in compliance with GLP. The high exposure concentration of 25.2 mg/m^3 induced substance-related effects which reflect an inflammatory response in the lung tissue with morphological tissue reaction. These tended to disappear during the recovery phase. The effects from a mild exposure concentration (5.39 mg/m^3) were confined to a very slight increase in the relative neutrophil count with a concomitant decrease in the relative macrophage count on the day after exposure, but these were only statistically significant in males. NOAEC could be defined as 5.39 mg/m^3 and assigned as category 2. Therefore, for specific target organ toxicity, repeated inhalation exposure, silicon dioxide can be assigned to category 2.

4.6.5 Genetic toxicity of silicon dioxide

Silicon dioxides (C and D) were tested for in vitro genotoxicity based on TG 471 and found to be negative for bacterial reverse mutation assay, with or without the presence of metabolic system. Chromosomal aberration tests conducted on silicon dioxides (A, C and D) were negative. In vitro mammalian cell micronucleus tests conducted on silicon dioxides (A, B, C and D) were positive for 9/25. In vitro comet assays conducted for silicon dioxides (A, B, C and D) were positive for 12/22.

In vivo genotoxic studies for silicon dioxides (A, B, C and D) based on TG 474 mammalian erythrocyte micronucleus tests, were positive for two only and for the lowest dose. In vivo DNA damage and/or repair mouse spot tests were all negative silicon dioxide (A, B, C and D).

Therefore, the silicon dioxides were not genotoxic, with strong evidence for in vitro and weak evidence for in vivo genotoxicity.

Table 4.6.2 Summary of genotoxicity of silicon dioxide (SiO_2)

NANO-MATERIAL	BACTERIAL REVERSE MUTATION ASSAY	CHROMO-SOMAL ABERRATION	IN VITRO MAMMALIAN CELL MICRONUCLEUS TEST	IN VITRO SINGLE CELL GEL/COMET ASSAY IN MAMMALIAN CELLS FOR DETECTION OF DNA DAMAGE	IN VIVO MAMMALIAN ERYTHROCYTE MICRONUCLEUS TEST,	DNA DAMAGE AND/OR REPAIR, MOUSE SPOT TEST
SiO2 (A)	ND	negative	negative, negative, negative, (+)	(+), (+), (+), (+)	negative, negative, negative	negative, negative
SiO2 (B)	ND	ND	negative, (+), negative, (+), negative, negative, (+), negative, (+), negative	negative, negative, negative, negative, negative, (+), negative, (+)	negative, negative, negative, negative, negative, negative	negative, negative, negative, negative
SiO2 (C)	negative	negative	negative, (+), negative, (+), negative	negative, (+), (+), (?)	negative, negative, (+) for the lowest dose only	negative, negative
SiO2 (D)	negative	negative	(?), (?), (?), (+), (+),negative	negative, (+), (+), (+), (+), (+)	negative, (+) for the lowest dose only	negative, negative

ND: not determined.

4.6.6 Reproductive and developmental toxicity of silicon dioxide

Two generation studies were conducted for silicon dioxide (A) using rats fed orally up to 1000 mg/kg bw/day. This had no adverse effect on the reproductive performance of the rats or on the growth and development of their offspring into adulthood; they were examined over two consecutive generations. One generation study was conducted for silicon dioxide (C) using oral feeding. Clinical symptoms were not observed in parents or during lactation; behavioural, developmental or structural abnormalities were not observed. Another generation study was conducted for silicon dioxide (D) using oral feeding. Clinical symptoms were not observed in parents or during lactation; behavioural, developmental or structural abnormalities were not observed.

4.6.7 Carcinogenicity of silicon dioxide

The carcinogenicity of silicon dioxide was not applicable.

The IARC evaluated amorphous silica as a group 3 carcinogen in 1998 (its carcinogenicity is not classified in humans). Within the amorphous silica group, synthetic amorphous silica is a fused silica and known as a nanomaterial. There is inadequate evidence available of the effects of synthetic amorphous silica on animals.

4.6.8 Hazard categorization for silicon dioxide

It is reported that repeated exposure to silicon dioxides may cause lung damage. Therefore for specific target organ toxicity, repeated inhalation exposure, silicon dioxide can be assigned to category 2 with strong evidence.

Table 4.6.3 Data quality of silicon dioxide studies

HEALTH HAZARDS	NO. OF STUDIES	NO. OF HIGH QUALITY (HAZARD CATEGORY)	NO. OF MEDIUM QUALITY (HAZARD CATEGORY)	NO. OF LOW QUALITY (HAZARD CATEGORY)	EVIDENCE GRADE
Acute toxicity (oral)	10	3 (all none)	7 (all none)	NA	Strong
Acute toxicity (dermal)	NA	NA	NA	NA	NA
Acute toxicity (inhalation)	7	7 (all none)	NA	NA	Strong
Skin corrosion/irritation	7	3 (all none)	4 (all none)	NA	Strong
Serious eye damage/eye irritation	5	1 (none)	4 (all none)	1 (none)	Strong
Respiratory or skin sensitization	3	1	NA	2	Strong
Genetic toxicity (in vitro)	64	10 (10–)	32 (32–)	22 (22–)	NA
Genetic toxicity (in vivo)	27	NA	11 (10–; 1+)	16 (15–; 1+)	Weak
Carcinogenicity	NA	NA	NA	NA	NA
Reproductive toxicity	3	1 (none)	2 (all none)	NA	Strong
Specific target organ toxicity single exposure	NA	NA	NA	NA	NA
Specific target organ toxicity repeated exposure (oral)	4	4 (all none)	NA	NA	Strong
Specific target organ toxicity repeated exposure (inhalation)	11	10 (7 category 2, 3 none)	1 (none)	NA	Strong

na: not applicable no studies

Table 4.6.4 Hazard categorization of silicon dioxide

HEALTH HAZARDS	HAZARD CATEGORY	EVIDENCE GRADE
Acute toxicity	No hazard categorization	Strong
Skin corrosion/irritation	No hazard categorization	Strong
Serious eye damage/eye irritation	No hazard categorization	Strong
Respiratory or skin sensitization	No hazard categorization	Strong
Germ cell mutagenicity	No hazard categorization	Weak
Carcinogenicity	No data to classify	NA
Reproductive toxicity	No hazard categorization	Strong
Specific target organ toxicity single exposure	No data to classify	NA
Specific target organ toxicity repeated exposure	Category 2 (inhalation): substance that, based on evidence from studies in experimental animals, can be presumed to have the potential to be harmful to human health	Strong

na: not applicable no studies

4.7 Titanium dioxide

4.7.1 Physicochemical properties of titanium dioxide

The titanium dioxide nanoparticles evaluated for hazard categorization are listed in Table 4.7.1. These particles were reference material or alternative reference materials for the sponsorship programme for the testing of manufactured nanomaterials. Their extensive physicochemical characterization data can be found in the following published dossiers (http://www.oecd.org/chemicalsafety/nanosafety/titanium-dioxide-nm100-nm105-manufactured-nanomaterial.htm).

Table 4.7.1 Physicochemical properties of titanium dioxide (TiO_2)

NANO-MATERIAL	DOSSIER NO.	REF ID[a]	MANUFACTURER	CHARACTERISTICS
Titanium dioxide	No 54. ENV/JM/MONO (2015)17/ Part 1, Part 2, Part 3, Part 4, Part 5, Part 6	A	Degussa/Evonik	Aeroxide P25
		B	Cristal Global	Tiona AT-1 (non-nano reference)
		C	Sachtleben	Hombikat UV 100 Average diameter < 10 nm (the representative TEM picture confirms the particle size provided by the supplier [8-10 nm])
		D	Cristal Global	PC105 Average diameter 22 nm (primary particle size measured by three instruments: 21 ± 10 nm, 22 ± 6 nm, 22 nm. Zeta size is 423.3 ± 59.4, intensity distribution main peak is 686.6 ± 40.6 nm, FWHM main peak is 414.1 ± 107.6 nm
		E	Sachtleben	UV TITAN M262. Average diameter 25 nm (the representative TEM picture confirms the particle size provided by the supplier [20 nm]). Zeta size is 113.2 ± 3.2, intensity distribution main peak is 138.4 ± 7.7 nm, FWHM main peak is 73.6 ± 11.0 nm
		F	Sachtleben	UV TITAN M212 Average diameter 22 nm (the representative TEM picture confirms the particle size provided by the supplier [20 nm]). Zeta size is 128.6 ± 1.3, intensity distribution main peak is 165.8 ± 5.9 nm, FWHM main peak is 89.0 ± 10.3 nm
		TiO_2^{NS}	Unknown	Not specified
		G	Unknown	Size unspecified; anatase; purity, 98%
		H	Unknown	Flat platelets Dimension, 10–35 µm; 28% titanium dioxide; 72% mica
		I	Degussa P25, Germany	MMAD, 0.80 µm
		J	Unknown	99.9% <0.5 µm; purity unspecified
		K	Unknown	Rutile 99% pure; MMAD, 1.5–1.7 µm; ~84% of dust particles <13 µm
		L	Unknown	Rutile 99.5%; MMAD, 1.1 µm
		M	Degussa P25 and P805	The first type was P25: hydrophilic, majority anatase; mean particle size, ~0.025 µm; density, 3.8 g/mL; specific surface area, 52 m^2/g. The second type was P805 (AL 90 003-2): hydrophobic, mean particle size 0.021 µm, density 3.8 g/mL, specific surface area 32.5 m^2/g. The third type was AL 23 203-3: hydrophilic, anatase; mean particle size ~0.2 µm; density 3.9 g/mL, specific surface area 9.9 m^2/g
		N	Unknown	Purity unspecified, particle size 97% <5 µm, 51% <0.5 µm)
		O	Unknown	Three preparations of titanium dioxide (>99% pure coated with antimony trioxide; >95% pure coated with aluminium oxide; or >85% pure coated with both compounds)
		P	Unknown	Purity >98%, manually ground
		Q	Unknown	Fibre length ~2.5 µm, fibre diameter ~0.125 µm, fibre concentration of titanium oxide whiskers was 639 × 10^3/µg

FWHM: full width at half maximum; MMAD:mass median aerodynamic diameter TEM:transmission electron microscopy

[a] The letters are used to identify the nanomaterials discussed in the text.

4.7.2 Acute toxicity of titanium dioxide

It is recommended that acute oral and dermal toxicities are tested based on TG 420 and 474 for oral, TG 402 for dermal, and TG 403 for inhalation. However, it was not clear whether these studies were conducted according to the test guidelines. Acute oral and dermal studies did not show any mortality up to 10 000 mg/kg/bw. Acute inhalation toxicity studies did not show any mortality up to 2.29 mg/m^3 air. Therefore, no acute toxicity hazard categorization was assigned for titanium dioxide nanoparticles.

4.7.3 Dermal and eye irritation/corrosion of titanium dioxide

It is recommended that dermal irritation/corrosion studies are based on TG 404 and TG 431. However, studies conducted by other methods were also listed. Titanium dioxide nanoparticles did not cause irritation and corrosion to the skin or eyes according to the results of tests carried out (Mizuno et al., 2011a; 2011c). Thus, titanium dioxide was not assigned for hazard categorization.

4.7.4 Sensitization of titanium dioxide

There was no toxicological report for classifying respiratory sensitization. According to human and guinea-pig data, titanium dioxide nanoparticles had no sensitizing effect on the skin and it was therefore not assigned for hazard categorization.

4.7.5 Specific target organ toxicity arising from repeated exposure to titanium dioxide

Titanium dioxide nanoparticles did not show any significant effects in 14-day repeated exposure studies at 6250, 12 500, 25 000, 50 000 or 100 000 mg/kg of oral feed (National Cancer Institute, 1979). Therefore, titanium dioxide nanoparticles could not be assigned to the specific target organ toxicity by orally repeated exposure.

Many studies indicated that titanium dioxide nanoparticles could be classified as specific target organ toxicity repeated exposure category 1. NM 105 could be classified as category 1 based on the inflammatory effect and histopathological findings on the lung (Ma-Hock et al., 2009; Creutzenberg et al., 1990, 2012, 2013; Oberdorster et al., 1994a, 1994b; Baggs et al., 1997; Bermudez et al., 2004, Muhle et al. 1990). NM 104 could also be classified as category 1. This was based on testing for repeated dose toxicity through inhalation subacutely according to TG 412, exposed up to 48 mg/m3 that showed effects on haematological and clinical endpoints, organ weights, gross pathology, and non-neoplastic histopathology (Creutzenberg et al., 2013).

Unknown titanium dioxide nanoparticles at a level of 3.2~20 mg/m^3 also caused focal septal thickening due to hypertrophy and hyperplasia of epithelial cells, and mononuclear cell infiltration by 8-month exposure (Takanaka et al., 1987). Aerosols of titanium dioxide nanoparticles also caused desquamation of the lung tissue, interstitial coniosis, bronochitis, and emphysema by 1-to-2 month exposure (Samoĭlova & Kireev, 1975).

In contrast, Trochimowicz et al. (1988) reported that the NOAEL for 4 weeks' repeated titanium dioxide nanoparticles was 1120 mg/m^3. Lee et al., (1985b) also reported that there were no abnormal clinical signs, body weight changes, or excess mortality, unchanged incidence and severity of neoplastic and non-neoplastic lesions. In summary, titanium dioxide nanoparticles can be assigned to specific target organ toxicity repeated exposure category 1 for inhalation exposure.

4.7.6 Genotoxicity of titanium dioxide

It is recommended that genotoxicity studies are conducted based on in vitro bacterial reverse mutation assay (based on TG 471), chromosomal assay (TG 473), in vivo micronucleus assays (TG 474), comet assay (TG 489), etc. However, various other methods were also applied when

studying the genotoxicity of titanium dioxide nanoparticles. Reverse mutation assay was performed in the in vitro bacteria system; chromosome aberration, the micronucleus test, single cell gel/comet assay, gene mutation assay, unscheduled DNA synthesis, sister chromatid exchange assay and bioluminescence assay were all performed in the in vitro cell culture system. In the in vivo system, rats and mice were mainly used. Micronucleus assay, single cell gel/comet assay, transgenic animal mutagenicity and somatic assay in Drosophila were studied.

The genotoxicity of TiO_2 (A) in vitro was not clear. It was positive in the alkaline comet assay (Kang et al. 2008) and other comet assays (Barillet et al., 2010; Jugan et al., 2012), Genotoxicity was also positive in the single cell gel/comet assay in Wistar rats (Fassard, 2013). However, negative results were also reported. Namely, the genotoxicity of TiO_2 (A) was negative in the bacterial reverse mutation assay (Mizuno, 2011b). The effects were also negative in the comet and micronucleus assays (Shi et al., 2009). Although the genotoxicity of TiO_2 (A) was positive in the colon and spleen of rats (Fassard, 2013), it was negative in the micronucleus assay and in vivo single cell gel/comet assay (Fassard, 2013).

The genotoxicity of TiO_2 (C) was positive in the in vitro comet and micronucleus assays (Gurr et al., 2005). However, TiO_2 (C) did not cause a positive effect in the single cell gene/comet assay and the micronucleus assay in Sprague-Dawley rats administrated intratracheally (Fassard, 2013).

Seven genotoxicity studies of TiO_2 (D) in the single cell gel/comet assay were positive from eight experiments, but one was positive in the micronucleus test from seven studies in the in vitro system (Norppa, 2013). The genotoxicity was negative in gene mutation. In the in vivo system, genotoxicity was positive in only one case in the single cell gel/comet assay out of three cases. Genotoxicity was negative in the micronucleus assay and transgenic animal mutagenicity assay.

Though two positive results were reported from six in vitro micronucleus tests, the genotoxicity of TiO_2 (E) was negative in the in vivo system (Norppa, 2013). The genotoxicity results of TiO_2 (F) were similar to those of TiO_2 (E).

There were more negative than positive genotoxic reports for titanium dioxide. In conclusion, titanium dioxide was not classified as a genotoxicant.

Table 4.7.2 Summary of genotoxicity (in vitro) of titanium dioxide (TiO2)

NANO-MATERIAL	BACTERIAL REVERSE MUTATION ASSAY	CHROMO-SOMAL ABERRATION	IN VITRO MICRONUCLEUS TEST	IN VITRO SINGLE CELL GEL/COMET (INCLUDING EFFECT ON THE DNA)	GENE MUTATION ASSAY	OTHER
TiO_2 (A)	Negative	No data	Negative, Weak positive, Positive	No data	No data	Negative, (+), (+), (+)
TiO_2 (B)	No data	No data	No data	No data	No data	No data
TiO_2 (C)	No data	No data	Positive	No data	No data	Positive
TiO_2 (D)	No data	No data	(-), (-), (-), (-), (-), Equivocal, Equivocal, Positive	Negative, Equivocal, Equivocal, (+), (+), (+), (+), (+)	Negative	No data
TiO_2 (E)	No data	No data	(-), (-), (-), (-) (+) , (+)	(-), (-), (-), (-), Equivocal, Equivocal,	Negative	No data
TiO_2 (F)	No data	No data	(-), (-), (-), (-) (+), (+)	(-), (-), (-), (-), (-), Equivocal	No data	No data
TiO_2 (NS)	(-), (-), (-), (-),	Negative	No data	No data	Negative	(-), (-), (-), (-), (-), (-)

Table 4.7.3 Summary of genotoxicity (in vivo) of titanium dioxide (TiO2)

NANO-MATERIAL	MICRONUCLEUS ASSAY	SINGLE CELL GEL/COMET ASSAY (INCLUDING DNA DAMAGE ASSAY)	TRANSGENIC ANIMAL MUTAGENICITY	SOMATIC ASSAY IN DROSOPHILA
TiO_2 (A)	(-), (-)	Negative Positive	No data	No data
TiO_2 (B)	No data	No data	No data	No data
TiO_2 (C)	Negative	Negative	No data	No data
TiO_2 (D)	(-), (-), (-)	Negative Positive	Negative	No data
TiO_2 (E)	(-), (-), (-)	(-), (-)	No data	No data
TiO_2 (F)	(-), (-), (-)	Negative Positive	No data	No data
TiO_2 (Not specified)	No data	Negative	No data	Negative

4.7.7 Reproductive and developmental toxicity of titanium dioxide

Scuri et al., (2010) reported that TiO_2 (A) caused age-dependent upregulation in the expression of lung neurotrophins associated with increased airway responsiveness in neonates and weanlings but not in adults. From this report, TiO_2 (A) could be classified as category 2. However, more study reports are needed to enable classification of reproductive and developmental toxicity.

4.7.8 Carcinogenicity of titanium dioxide

The IARC conducted carcinogenicity evaluations for pigmentary and ultrafine titanium dioxide in 2006. The studies were carried out via intratracheal administration in hamsters, female rats and mice; by subcutaneous injection in rats; and by intraperitoneal administration in male mice and female rats. In one inhalation study, the incidence of benign and malignant lung tumours was seen to increase in female rats. In another inhalation study, the incidence of benign lung tumours increased in the high-dose groups of male and female rats. Cystic keratinizing lesions that were diagnosed as squamous-cell carcinomas but re-evaluated as non-neoplastic pulmonary keratinizing cysts, were also observed in the high-dose groups of female rats. Two inhalation studies in rats and one in female mice gave negative results. Female rats intratracheally-instilled with two types of titanium dioxide, showed an increased incidence of both benign and malignant lung tumours following treatment. Tumour incidence did not increase in intratracheally-instilled hamsters and female mice. Oral, subcutaneous and intraperitoneal administration did not produce a significant increase in the frequency of any type of tumour in mice or rats (IARC monograph 93, 2010). Thus titanium dioxide can be assigned to carcinogenicity IARC category 2B.

4.7.9 Other studies of titanium dioxide

No data are available to assess the effects of titanium dioxide on specific target organ toxicity (single exposure) and as an aspiration hazard.

4.7.10 Hazard categorization of titanium dioxide

Titanium dioxide can probably not be classified as a genotoxicant even though this may be a controversial view. Until now, the reproductive toxicity and specific target organ toxicity (single exposure) of titanium dioxide could not be classified. It is reported that repeated exposure to titanium dioxide may cause lung damage and therefore that it can be assigned to specific target organ toxicity repeated exposure (inhalation) category 1, with moderate evidence.

Table 4.7.4 Data quality of titanium dioxide studies

HEALTH HAZARDS	NO. OF STUDIES	NO. OF HIGH QUALITY (HAZARD CATEGORY)	NO. OF MEDIUM QUALITY (HAZARD CATEGORY)	NO. OF LOW QUALITY (HAZARD CATEGORY)	EVIDENCE GRADE
Acute toxicity (oral)	8	NA	3 (all none)	5 (all none)	Moderate
Acute toxicity (dermal)	2	NA	2 (all none)	NA	Moderate
Acute toxicity (inhalation)	3	NA	1 (none)	2 (all none)	Moderate
Dermal corrosion/irritation	10	NA	6 (all none)	4 (all none)	Moderate
Eye corrosion/irritation	3	NA	2 (all none)	1 (none)	Moderate
Skin sensitization	2	NA	2 (all none)	NA –	Moderate
Specific target organ toxicity from repeated exposure (oral)	2	NA	NA	2 (none, unclassifiable)	Weak
Specific target organ toxicity from repeated exposure (inhalation)	40	3 (2 category 1, 1 unclassifiable)	34 (10 category 1, 2 category 2, 2 none, 20 unclassifiable)	4 (2 category 2, 2 unclassifiable)	Strong
Specific target organ toxicity from repeated exposure (dermal)	1	NA	NA	1 (unclassifiable)	Weak
Genetic toxicity (in vitro)	62	24	37	1	Not evaluated
Genetic toxicity (in vivo)	28	12 (all none)	16 (5 category 2, 11 none)	NA	Strong
Reproductive toxicity	1	NA	NA	1 (category 2)	Weak
Carcinogenicity	NA	NA	NA	NA	NA

Table 4.7.5 Hazard categorization of titanium dioxide

HEALTH HAZARDS	HAZARD CATEGORY	EVIDENCE GRADE
Acute toxicity	No hazard categorization	Moderate
Skin corrosion/irritation	No hazard categorization	Moderate
Serious eye damage/eye irritation	No hazard categorization	Moderate
Respiratory or skin sensitization	No hazard categorization	Moderate
Germ cell mutagenicity	No hazard categorization	Strong
Carcinogenicity	No data to classify #IARC group 2B	NA
Reproductive toxicity	Category 2	Weak
Specific target organ toxicity single exposure	No data to classify	NA
Specific target organ toxicity repeated exposure	Category 1(inhalation); substance that produced significant toxicity in humans, or that was expected to do so, based on evidence from studies in experimental animals	Strong

IARC: International Agency for Research on Cancer.

4.8 Cerium dioxides

4.8.1 Physicochemical properties of cerium dioxides

The cerium dioxides evaluated for hazard categorization are listed in Table 4.8.1. Cerium dioxides were reference material, or alternative reference materials, for the sponsorship programme for the testing of manufactured nanomaterials. Their extensive physicochemical characterization data can be found in the following published dossiers (http://www.oecd.org/chemicalsafety/nanosafety/cerium-oxide-manufactured-nanomaterial.htm).

Table 4.8.1 Physicochemical properties of cerium dioxide (CeO2)

NANOMATERIALS	DOSSIER	MANUFACTURER	CHARACTERISTICS
Cerium dioxide NW-211 (A)[a] Cerium dioxide NW-212 (B) Cerium dioxide NW-213 (C)	No 45. ENV/JM/MONO (2015)8	Ceria dry CeO_2 material Antaria, Australia	Agglomerated/aggregated structure, spherical particle Primary particle size by TEM 10 nm Agglomerates particle size mean 293 nm Zeta potential 28 mV DI water
		Nangrain CeO_2 material Umicore, Belgium	Agglomerated/aggregated structure, geometrical shaped primary particles Primary particle size by TEM 24 nm Agglomerates particle size mean 213 nm Zeta potential 33 mV DI water
		Micron CeO_2 material Sigma Aldrich, Australia	Agglomerated/aggregated structure, irregular shapes Primary particle size by TEM 54 nm Agglomerates particle size mean 349 nm Zeta potential -7 mV DI water

DI: deionized water; TEM: transmission electron microscopy

[a] The letters in this column are used to identify the nanomaterials discussed in the text.

4.8.2 Acute toxicity of cerium dioxide

The inhalation toxicity of cerium dioxide has been studied in experimental animals. There was no mortality in the study of acute inhalation toxicity.

4.8.3 Specific target organ toxicity arising from repeated exposure to cerium dioxide

Ceria dry CeO_2 increased neutrophil content and biochemical parameters in bronchoalveolar lavage fluid by 28 days of inhalation and NOAEL was suggested to be 1.2mg/m^3. Ceria dry CeO_2 was evaluated as category 1 in specific target organ toxicity with moderate evidence.

4.8.4 Hazard categorization for cerium dioxide

Data for cerium dioxide were available for acute toxicity (inhalation) and specific target organ toxicity repeated exposure (inhalation). The data showed that repeated exposure to cerium dioxides may cause lung damage and it can therefore be assigned to specific target organ toxicity repeated exposure (category 1) with moderate evidence.

Table 4.8.2 Data quality of cerium dioxide acute toxicity studies

HAZARD CATEGORY	NO. OF STUDIES	NO. OF HIGH QUALITY	NO. OF MEDIUM QUALITY	NO. OF LOW QUALITY	EVIDENCE GRADE
Acute toxicity (inhalation)	2	NA	NA	2 (all none)	Weak
Specific target organ toxicity from repeated exposure (inhalation)	3	3 (1 category 1, 2 none)	NA	NA	Strong → Moderate

Table 4.8.3 Hazard categorization of cerium dioxide

HEALTH HAZARD	HAZARD CATEGORY	EVIDENCE GRADE
Acute toxicity	No hazard categorization	Weak
Skin corrosion/irritation	No data to classify	NA
Serious eye damage/eye irritation	No data to classify	NA
Respiratory or skin sensitization	No data to classify	NA
Germ cell mutagenicity	No data to classify	NA
Carcinogenicity	No data to classify	NA
Reproductive toxicity	No data to classify	NA
Specific target organ toxicity single exposure	No data to classify	NA
Specific target organ toxicity repeated exposure	Category 1 (inhalation); substance that produced significant toxicity in humans, or that is likely to do so, based on evidence from studies in experimental animals	Moderate

4.9 Dendrimers

The dendrimers evaluated for hazard categorization are listed in Table 4.9.1. Their extensive physicochemical characterization data can be found in the following published dossiers (http://www.oecd.org/chemicalsafety/nanosafety/dendrimers-manufacturednanomaterial.htm).

Table 4.9.1 Physicochemical properties of dendrimers

NANO-MATERIAL	DOSSIER NO.	MANUFACTURER	CHARACTERISTICS
Polyamidoamine (PAMAM) Dendrimers	No 46. ENV/JM/MONO(2015)9	Not known	Size: G3-PAMAM-$(NH_2)_{32}$ 2-4 nm Aggregates 100-200 nm G4-PAMAM-$(NH_2)_{32}$ 3-5 nm Aggregates 100–300 nm Partition coefficient: -1.48 to -2.39 at pH 7.4 Zeta potential: G3-PAMAM-$(NH_2)_{32}$ f -4.07 ± 2.85 mV G4-PAMAM-$(NH_2)_{32}$ –5.12 ± 4.12 mV

There were no data on animal studies or genotoxicity and therefore no hazard categorization could be made.

4.10 Nanoclays

The nanoclays evaluated for hazard categorization are listed in Table 4.10.1. Their extensive physicochemical characterization data can be found in the following published dossiers (http://www.oecd.org/chemicalsafety/nanosafety/nanoclays-manufacturednanomaterial.htm).

Table 4.10.1 Physicochemical properties of nanoclays

NANOMATERIALS	DOSSIER	MANUFACTURER	CHARACTERISTICS
Bentonite Hydrated sodium calcium Aluminium Magnesium Silicate hydroxide	No 47. ENV/JM/MONO(2015)10	Not known	Size: mean Feret's diameter 39.5 µm ± 23.8 µm by SEM Size in de-ionized water 288 nm Specific surface area: mean 51.9 m^2/g Standard deviation: 1.6 m^2/g Surface chemistry: NM-600 (0153) Bentonite sample contains: calcium, iron, sodium, magnesium, aluminium, silicon and sulfur by SEM-EDX

SEM: scanning electron microscopy SEM-EDX: SEM with Energy Dispersive X-Ray Analysis (EDX).

There were no data on animal studies or genotoxicity and therefore no hazard categorization could be made.

4.11 Zinc oxide

4.11.1 Physicochemical properties of zinc oxide

Zinc oxides evaluated for hazard categorization are listed in Table 4.11.1.

Several nanoscale zinc oxides with coating or without coating, and non-nanoscale zinc oxides were reference material for the sponsorship programme for the testing of manufactured nanomaterials. Their extensive physicochemical characterization data can be found in the following published dossiers (http://www.oecd.org/chemicalsafety/nanosafety/zinc-oxide-manufactured-nanomaterial.htm).

Table 4.11.1 Physicochemical properties of zinc oxide (ZnO)

NANOMATERIALS	DOSSIERS	MANUFACTURER	CHARACTERISTICS
NM-110, Z-COTE, zinc oxide nano uncoated, purity 99%	No 52. ENV/JM/MONO(2015) Part1, Part2, Part3	BASF SE	77.5 ± 18 nm, specific surface area 6.6 ± 0.3 (m^2/g)
NM-111, Z-COTE HP1, zinc oxide nano coated triethoxycaprylylsilane (2%), purity 96%		BASF SE	75.2 ± 7.6 nm, specific surface area 11.8 ± 0.2 (m^2/g)
NM-112, zinc oxide nano, purity 99.5%		BASF SE	33.75 ± 6.2 nm, specific surface area 25.9 ± 0.3 (m^2/g)
NM-113, zinc oxide uncoated non-nanosized ZnO		BASF SE	149.7 ± 25 nm, specific surface area 4.0 ± 0.15 (m^2/g)

4.11.2 Acute toxicity of zinc oxide

Acute oral toxicity was evaluated by administering zinc oxide, similar to NM-112, orally up to 5000 mg/kg based on OECD TG 401 (Wang et al., 2008). LD 50 for Z-COTE HP1 (NM-111) was > 5000 kg/bw. Acute oral toxicity could not be categorized. Acute dermal toxicity was < 2000 mg/bw resulting in no classification.

4.11.3 Zinc oxide dermal and eye irritation/corrosion

A dermal irritation/corrosion study for Z- COTE HP1 (NM-111) using in vitro skin corrosion and human skin model tests, showed it was non-corrosive to the skin. Z- COTE (NM-110) was tested for eye irritation/corrosion using both ex vivo eye corrosion: Bovine corneal opacity and permeability tests and in vitro eye irritation: the EpiOcular test. Both were negative for serious eye damage.

4.11.4 *Specific target organ toxicity arising from repeated exposure to zinc oxide*

The comparative toxicity study of zinc oxide as coated or uncoated (similar to NM-113), as a pigment form or as zinc sulfate, showed no effect at the end of the observation period of 4 weeks, when injected intravenously at dose levels of 1 or 5 mg/kg bw. No hazard categorization can be made from this result.

The coated nanoscale (Z-COTE HP1) and non-coated microscale zinc oxide caused comparable and reversible histopathological findings restricted to the respiratory tract. The retained material was completely solved and eliminated rapidly since no increased zinc contents were detected in any body compartment after the post-exposure period. The status of the respiratory burst of alveolar macrophages was temporarily increased by treatment with nano- and microscale zinc oxide and was fully reversible for Z-COTE HP1. Markers for cellular damage and inflammation were reversibly increased to a higher extent by microscaled zinc oxide than by Z-COTE HP1. Based on the results of the present study the NOAEC for Z-COTE HP1 was assessed as 1.5 mg/m3. Thus Z-COTE HP1 can be assigned to specific target organ toxicity repeated exposure category 1.

4.11.5 *Genotoxicity of zinc oxide*

In vitro genotoxicity tests for various zinc oxides were performed using the in vitro bacteria system based on OECD TG 471; the in vitro mammalian cell gene mutation test based on TG 476 and the chromosome aberration test based on OECD TG 473. They showed negative for the Ames and chromosomal aberration tests and equivocal for the mammalian cell gene mutation test. In vivo genotoxicity tests such as the micronucleus test which exposes zinc oxide by intraperitonela administration or inhalation; the comet assay; and immunohistochemical detection of oxidative DNA damage exposing zinc oxide by inhalation; were all negative

Table 4.11.2 Summary of zinc oxide genotoxicity

ZINC OXIDES	BACTERIAL REVERSE MUTATION ASSAY	CHROMO-SOMAL ABERRATION	IN VITRO MAMMALIAN CELL MICRONUCLEUS TEST	IN VIVO MAMMALIAN ERYTHROCYTE MICRONUCLEUS ASSAY	DNA DAMAGE AND/ OR REPAIR, IMMUNO-HISTOCHEMICAL DETECTION OF OXIDATIVE DNA DAMAGE
Z-COTE (NM-110);	No data	Negative	Equivocal	Negative	Negative
Z-COTE HP1 (NM-111);	No data	Negative	Equivocal	Negative	Negative
Zinc oxide, (NM-113)	Negative	No data	Equivocal	Negative	Negative
Z-COTE MAX, size (nm): < 200, specific surface area 12–24 m^2/g	Negative	No data	No data	Negative	Negative

ND: not determined.

4.11.6 *Reproductive and developmental toxicity of zinc oxide*

Z-COTE HP1(NM-111) zinc oxide was tested for reproductive and developmental toxicity based on OECD TG 414. Animals were exposed inhalationally to Z-COTE HP1 at concentrations of 0.3, 1.5 and 7.5 mg/m^3 for 14 consecutive days from implantation to one day prior to the expected day of parturition. A dose of 7.5 mg/m^3 caused moderate alveolar lipoproteinosis and slight inflammation to maternal lungs. These histopathologic findings are regarded to be adverse in nature. There were no adverse fetal findings evident at any dose for developmental toxicity.

4.11.7 Hazard categorization of zinc oxide

Repeated exposure to zinc oxide via inhalation may cause lung damage and therefore it can be assigned to specific target organ toxicity repeated exposure (inhalation) category 1 with moderate evidence.

Table 4.11.3 Data quality of zinc oxide studies

HEALTH HAZARDS	NO. OF STUDIES	NO. OF HIGH QUALITY (HAZARD CATEGORY)	NO. OF MEDIUM QUALITY (HAZARD CATEGORY)	NO. OF LOW QUALITY (HAZARD CATEGORY)	EVIDENCE GRADE
Acute toxicity (oral)	1	NA	1 (none)	NA	Moderate
Acute toxicity (dermal)	1	1 (none)	NA	NA	Strong
Dermal corrosion/irritation	1	1 (none)	NA	NA	Strong
Eye corrosion/irritation	2	NA	2 (all none)	NA	Moderate
Skin sensitization	NA	NA	NA	NA	NA
Specific target organ toxicity from repeated exposure (inhalation)	5	2 (1 category 1, none)	2 (all none)	1 (none)	Strong → Moderate
Genetic toxicity (in vitro)	4	4	NA	NA	Not evaluated
Genetic toxicity (in vivo)	4	2 (all none)	1 (none)	1 (none)	Strong
Reproductive toxicity	1	1 (none)	NA	NA	Strong
Carcinogenicity	NA	NA	NA	NA	NA

Table 4.11.4 Hazard categorization of zinc oxide

HEALTH HAZARDS	HAZARD CATEGORY	EVIDENCE GRADE
Acute toxicity	No hazard categorization for oral No hazard categorization for dermal	Moderate Strong
Skin corrosion/irritation	No hazard categorization	Strong
Serious eye damage/eye irritation	No hazard categorization	Moderate
Respiratory or skin sensitization	No data to classify	NA
Germ cell mutagenicity	No hazard categorization	Strong
Carcinogenicity	No data to classify	NA
Reproductive toxicity	No hazard categorization	Strong
Specific target organ toxicity single exposure	No data to classify	NA
Specific target organ toxicity repeated exposure	Category 1(inhalation); substance that produced significant toxicity in humans, or that is likely to do so, based on evidence from studies in experimental animals	Moderate

4.12 Physical hazards of manufactured nanomaterials

The OECD dossiers on nanomaterials had sections relating to physical hazards including flash points, auto-flammability, flammability, explosiveness and oxidizing properties. Most nanomaterials are not tested for physical hazards. Only titanium dioxide was tested for flammability and explosiveness and the results indicated that it was neither (Bresh et al., 2012).

Table 4.12.1 Summary table for physical hazards of nanomaterials

NANO-MATERIAL	FLASH POINT	AUTO-FLAMMABILITY	FLAMMABILITY	EXPLOSIVENESS	OXIDIZING PROPERTIES
Fullerene	Not tested	Not tested	Not tested	Not tested	Not tested
SWCNT	Not tested	Not tested	Not tested	Not tested	Not tested
MWCNT	Not tested	Not tested	Not tested	Not tested	Not tested
Silver nanoparticles	Not tested	Not tested	Not tested	Not tested	Not tested
Gold nanoparticles	Not tested	Not tested	Not tested	Not tested	Not tested
Silicon dioxide	Not tested	Not tested	VDI 2263-1 Material does not catch fire. "Brennzahl" (BZ) 1	No risk of dust explosion	Not tested
Titanium dioxide (TiO2)	Not tested	Not tested	NM 105, TiO_2 (P25) is not dust explosible and the burning behaviour corresponds to Burning Class 1 (no ignition); (Bresh et al., 2012)	NM 105, TiO_2 (P25) there is no ignition or explosion (Bresh et al., 2012)	Not tested
Cerium dioxide	Not tested	Not tested	Not tested	Not tested	Not tested
Dendrimer	Not tested	Not tested	Not tested	Not tested	Not tested
Nanoclay	Not tested	Not tested	Not tested	Not tested	Not tested
Zinc oxide	Not tested	Not tested	Not tested	Not tested	Not tested

MWCNT: multi-walled carbon nanotubes; SWCNT: single-walled carbon nanotubes; Brennzahl indicator of flammability ranging from 1 to 6 from no to very flammable.

5. Discussion

In this systemic review 11 nanomaterials were given hazard categorization based on information in the dossiers produced by the OECD Working Party on Manufactured Nanomaterials' Safety Testing Sponsorship Programme. The nanomaterials were fullerene, SWCNT, MWCNT, silver nanoparticles, gold nanoparticles, silicon dioxide, titanium dioxide, cerium oxide, dendrimer, nanoclay and zinc oxide.

The hazard categorization of nanomaterials is based on guidelines outlined in the United Nations GHS. Nanomaterials are assigned to nine hazard categories: acute toxicity, skin corrosion/irritation, serious eye damage/eye irritation, respiratory or skin sensitization, germ cell mutagenicity, carcinogenicity, reproductive toxicity, specific target organ toxicity (single exposure) and specific target organ toxicity (repeated exposure).

The data in the dossiers were evaluated using the criteria presented in the methods section of this paper based on OECD test guidelines, compliance with GLP guidelines and peer-reviewed publications. Many studies had conducted tests using the OECD guidelines with or without compliance to GLP. Some test were also published in the form of peer-reviewed papers. Other tests were not based on the guidelines but were presented as a peer-reviewed paper.

The OECD dossiers classified reliability into three categories: (1) reliable without restriction; (2) reliable with restriction; and (4) not assignable. We used the following terms to reflect these criteria: high (H), medium (M) and low (L) reliability. We also considered the number of reliable tests when weighing up the evidence and used the evidence grades: strong, moderate and weak.

A summary of the hazard categorizations for the 11 nanomaterials is presented in Table 5.1. No test data were available for several hazard areas such as carcinogenicity, target organ toxicity (single exposure), germ cell mutagenicity and reproductive toxicity. Despite the lack of data we have categorized as much as we can, based on information in the OECD dossiers.

There is not enough data available on fullerene to be able to make a hazard categorization. SWMCTs were assigned to category 2 for germ cell mutagenicity with low evidence, and to category 1 specific target organ toxicity (repeated inhalation exposure) with low evidence. MWCNTs were assigned as: category 2 for serious eye damage/eye irritation with strong evidence; category 2 for germ cell mutagenicity with strong evidence, and category 1 for specific target organ toxicity by repeated inhalation exposure, with moderate evidence. Some MWCNTs such as MWCNT-7 are IARC group 2B and GHS category 2B carcinogens.

Silver nanoparticles were assigned as category 1B skin sensitizers with strong evidence; category 1 for specific target organ toxicity by repeated inhalation exposure with moderate evidence, and category 2 for specific target organ toxicity by oral exposure with moderate evidence.

Only limited toxicological data are available for gold nanoparticles. They can be assigned as category 1 specific target organ toxicity by repeated inhalation exposure with strong evidence. There are many toxicity data available for silicon dioxide and titanium dioxide. Silicon dioxide is assigned as category 2 for specific target organ toxicity by repeated inhalation exposure, with strong evidence. Titanium dioxide was assigned as category 1 by specific target organ to toxicity (repeated inhalation) with strong evidence. Bulk titanium dioxide was categorized as IARC group 2B (IARC monograph, 2010). Many forms of titanium dioxide reviewed by the IARC were nanoscale in primary particle, thus nano titanium dioxide could be categorized as IARC 2B.

Cerium oxide can be assigned as category 1 specific target organ toxicity by repeated inhalation exposure with moderate evidence. Although there are dossiers for dendrimers and nanoclays, there were no hazard data to evaluate. Various forms of zinc oxides, both coated and non-coated, were evaluated in the OECD dossiers. Zinc oxide can be assigned as category 1 specific target organ toxicity by repeated inhalation exposure with moderate evidence.

Taken together, most nanomaterials that are used and manufactured in the workplace have specific target organ toxicity by repeated inhalation exposure. Some nanomaterials such as titanium dioxide and MWCNT-7 could be carcinogenic. Workers' exposure to MWCNTs should be minimized to avoid eye irritation. Workers should be aware of skin sensitization risk when they are handling silver nanoparticles.

Table 5.1 Hazard categorization of manufactured nanomaterials

MANUFACTURED NANO-MATERIALS	ACUTE TOXICITY	SKIN CORROSION/ IRRITATION	SERIOUS EYE DAMAGE/EYE IRRITATION	RESPIRATORY OR SKIN SENSITIZATION	GERM CELL MUTAGENICITY	CARCINO-GENICITY	REPRODUCTIVE TOXICITY	SPECIFIC TARGET ORGAN TOXICITY (SINGLE EXPOSURE)	SPECIFIC TARGET ORGAN TOXICITY (REPEATED EXPOSURE)
Fullerene	No hazard (strong E)	No hazard (moderate E)	No hazard (moderate E)	No hazard (moderate E)	No hazard (moderate E)	No data	No data	No data	No hazard (moderate E)
SWCNT	No hazard for oral (strong E) No hazard for Inhalation (moderate E)	No hazard (strong E)	No hazard (strong E)	No hazard (strong E)	Category 2 hazard (Weak E)	No data	Ambiguous (Weak E)	No data	Category 1 hazard for inhalation (Weak E)
MWCNT	No hazard (strong E)	No hazard (strong E)	Category 2 hazard (strong E)	No hazard (moderate E)	Category 2 hazard (strong E)	Category 2 hazard (moderate E) #IARC Mitzi-7 CNT 2B	No hazard (moderate E)	No data	Category 1 hazard for inhalation (moderate E) No hazard for oral (strong E)
Silver nanoparticles	No hazard (strong E)	No hazard (strong E)	No hazard (strong E)	Category 1B hazard (moderate E)	No hazard (strong E)	No data	No hazard (strong E)	No data	Category 1 hazard for inhalation (strong E) Category 2 hazard for oral (moderate E)
Gold nanoparticles	No data	No data	No data	No data	No data	No data	No data	No data	Category 1 hazard for inhalation (strong E)
Silicon dioxide	No hazard (strong E)	No hazard (strong E)	No hazard (strong E)	No hazard (strong E)	No hazard (Weak E)	No data	No hazard (strong E)	No data	Category 2 hazard for inhalation (strong E)
Titanium dioxide	No hazard (moderate E)	No hazard (moderate E)	No hazard (moderate E)	No hazard (moderate E)	No hazard (strong E)	No data #IARC 2B	Category 2 (weak E)	No data	Category 1 hazard for inhalation (strong E)
Cerium oxide	No hazard (weak E)	No data	No data	No data	No data	No data	No data	No data	Category 1 hazard for inhalation (moderate E)
Dendrimer	No data	No data	No data	No data	No data	No data	No data	No data	No data
Nanoclay	No data	No data	No data	No data	No data	No data	No data	No data	No data
Zinc oxide	No hazard for oral (moderate E) No hazard for dermal (strong E)	No hazard (strong E)	No hazard (moderate E)	No data	No hazard (strong E)	No data	No hazard (strong E)	No data	Category 1 hazard for inhalation (moderate E)

E: evidence grade; IARC: International Agency for Research on Cancer; no hazard: no hazard categorization.

6. Summary of findings of manufactured nanomaterials hazard category classification

Systematic review question: Which hazard classes can be assigned to specific MNMs according to the UN GHS and making use of MNM-specific dossiers as developed by the OECD? The MNM dossiers compiled by the OECD give an overview of the available toxicological data for a number of specific MNMs.

Evidence summary

Number of studies and participants

There were 11 OECD dossiers containing toxicity testing information. These were used by the systematic review team to assign one or more hazard classes, according to the GHS, to the following nanomaterials: fullerene, single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT), silver, gold, silicon dioxide, titanium dioxide, cerium dioxide, dendrimer, nanoclay and zinc oxide in nanoparticle form. For the assessment of carcinogenicity, the review team also used the evidence summaries compiled by IARC on SWCNTs, MWCNTs and titanium dioxide.

Data in the dossiers

Dossiers mostly contained results of in vivo animal studies and in vitro genotoxicity studies supplied by member countries and nongovernmental organizations such as the Business and Industry Advisory Committee to the OECD.

Risk of bias in the included dossiers

The main limitations to the studies included in the dossiers were that they did not fulfil the OECD criteria for good methodological quality, such as being published in a peer-reviewed journal and complying with good laboratory practice (GLP). For some studies, the GLP test data were not fully disclosed because of the company's intellectual property rights. Studies were classified at low risk of bias if they were in the OECD category 1 or 2, complied with GLP, were based on test guidelines and resulted in a peer-reviewed publication; at medium risk of bias if the above applied but there was no compliance with GLP; and at high risk of bias if none of the above applied.

Classification of MNMs

The MNMs were classified as having a specific hazard according to the GHS, having no hazard according to the available studies, or as having no data when these were not available for classification. "No hazard" does not necessarily imply that there is no hazard but only that this was not found in the studies used in the OECD dossiers.

For fullerene, there was evidence that there is no hazard for acute toxicity, skin-, eye- or respiratory damage, germ cell mutagenicity or specific target organ toxicity after repeated exposure but, for the other hazard classes, data were missing.

For SWCNT, there was evidence of a hazard for germ cell mutagenicity (Cat 2) and specific organ toxicity after repeated exposure (Cat 1). For reproductive toxicity no clear hazard could be established based on the available data. There was also evidence of no hazard in acute toxicity,

skin damage, respiratory/skin sensitization, or reproductive toxicity. For specific target toxicity after single exposure, there were no data. For carcinogenicity there were no data but there is an IARC classification 3, meaning not classifiable.

For MWCNT, there was evidence of a hazard for eye damage (Cat 2), germ cell mutagenicity (Cat 2), carcinogenicity (Cat 2, IARC 2B/3) and specific organ toxicity after repeated exposure (Cat 1). There was also evidence of no hazard in acute toxicity, skin damage, respiratory/skin sensitization, or reproductive toxicity. For specific target toxicity after single exposure, there were no data.

For silver nanoparticles, there was evidence of a hazard for respiratory/skin sensitization (Cat 1B) and specific target organ toxicity after repeated exposure (Cat 1–2). For acute toxicity, skin corrosion, eye damage, germ cell mutagenicity and reproductive toxicity there was evidence of no hazard. For carcinogenicity and specific target organ toxicity after single exposure, there were no data.

For gold nanoparticles, there was evidence for specific target organ toxicity after repeated exposure (Cat 1). There were no data for the other classes.

For silicon dioxide, there was evidence for specific target organ toxicity after repeated exposure (Cat 2), but no hazard for acute toxicity, skin or eye damage, respiratory or skin sensitization, germ cell mutagenicity and reproductive toxicity. For carcinogenicity and specific organ toxicity after single exposure, there were no data.

For titanium dioxide, there was evidence for possible carcinogenicity (IARC Cat 2B), reproductive toxicity (Cat 1), and specific organ toxicity after repeated exposure (Cat 1), but also evidence of no hazard for acute toxicity, skin or eye damage, respiratory or skin sensitization or germ cell mutagenicity. There were no data for specific organ toxicity after single exposure.

For cerium dioxide, there was evidence of specific target organ toxicity after repeated exposure (Cat 1), but also evidence of no hazard for acute toxicity. There were no data for the other hazard classes.

For dendrimer and nanoclay, there were no animal toxicity or genotoxicity data to use for classification.

For zinc oxide, there was evidence for specific organ toxicity after repeated exposure (Cat 1) but also evidence of no hazard for acute toxicity, skin or eye damage, germ cell mutagenicity and reproductive toxicity. There were no data for respiratory/skin sensitization, carcinogenicity and specific organ toxicity after single exposure.

For physical hazards, there was evidence that silicon dioxide and titanium dioxide were not flammable or explosive. There was no evidence for the other MNMs.

Quality of the evidence

The evidence was rated as strong if there was at least one study at low risk of bias; as moderate quality if there was at least one moderate-quality study; and as low quality if there were only studies at high risk of bias. The quality of the evidence for all but one of the classifications of hazards was in the moderate or strong category.

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