Report on nano-specific sex differences to direct future hazard assessment approaches

DELIVERABLE 5.3

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Abstract

Sex/gender-related differences in human response to external stressors and xenobiotics, including engineered nanomaterials (ENMs) alter all aspects of risk assessment (RA), not just toxicology, but also psychosocial and economic questions and problems. Therefore, meta-analysis of existing scientific data on the sex differences in ENMs toxicity has been foreseen as one of the main objectives of WP5, Task 5.3. The Deliverable (D) 5.3. presents main results of this meta-analysis, while detailed analysis of a whole set of data has been used for preparation of two opinion papers on the topic of nano-specific sex differences. This work supports development of an improved, robust, predictive and nano-specific framework of regulatory-oriented approaches for human hazard assessment. The D5.3. and resulting papers direct future RA practices for ENMs by integrating sex analysis into the design of research methodology.

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TABLE OF CONTENTS

Contents

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List of Abbreviations

ADME - Absorption, Distribution, Metabolism and Excretion

Cl - Renal Clearance

D - Deliverable

EAB – External Advisory Board

EC – European Commission

ECG - electrocardiogram

ENMs – Engineered nanomaterials

ETAN - European Technology Assessment Network

ETPN - Nanomedicine European Technology Platform

GFR - glomerular filtration rate

OAT - Organic Anion Transporters

OECD – Organisation for Economic Corporation and Development

PFOA - Perfluorooctanoic Acid

R&I - Research and Innovation

RA – Risk Assessment

RGC – Risk Governance Council

SABV - Sex As a Biological Variable

SOP(s) – Standard Operating Procedure(s)

TD - Toxicodynamics

TK - Toxicokinetics

UGT - Uridine Diphosphate Glucoronosyltransferase

US - United States

US NIH - US National Institutes of Health

Vd - Volume of distribution

WP – Work package

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1. Introduction

Following raising awareness of difference in biological response between sexes and efforts of the European Commission (EC) to develop the sex/gender dimension in EU research,[1] RiskGONE project has incorporated the sex difference dimension into the work planned by WP5 by using *in vivo* data from animals of both sexes, by addressing sex differences in sensitivity, sex-specific impact of ENMs on biomarkers and sex-specific health-based guidance values. From a scientific point of view, sex/gender are specifically considered in the WP5 and WP6 tasks, respectively, and deliverables (D) 5.3. and 6.6. are expected to direct future RA approaches on nano-specific sex/gender differences.

Sex/gender effects are wide-ranging, operating at all biological levels, from gene expression to whole organism. While term "sex" refers to the biological attributes and distinguishes an organism as male, female, intersex or hermaphrodite, term "gender" is characterized by psychological, social and cultural factors shaping behaviour, attitude, stereotypes, technology and knowledge. Such differences were documented by many research studies and led to policy change at major funding bodies a decade ago including EC, US National Institutes of Health, Canadian Institutes of Health Research and many others. It has been well recognized that interpretation, validation, reproducibility and impact of research findings critically depend on the sex and gender analysis.[2]

More than a decade ago, in 2011, the Directorate-General for Research & Innovation of the EC convened an Expert Group "Innovation through Gender" within its work programme Science in Society of the Seventh Framework Programme for Research and Technological Development (EU FP7),[3] but it was initiated at Stanford University in July 2009. The Expert Group aimed to provide scientists and engineers with practical methods for sex/gender analysis, and to develop case studies as concrete illustrations of how sex/gender analysis leads to new ideas and excellence in research. The Expert Group provides globally accessible and peer-reviewed gendered innovations websites:

- http://ec.europa.eu/research/science-society/
- http://genderedinnovations.stanford.edu/

Main aims of gendered innovations are to add values to:

- research and engineering by ensuring excellence and quality in outcomes and enhancing sustainability,
- society by making research more responsive to social needs, and
- business by developing new ideas, patents, and technology.

[1] https://ec.europa.eu/info/research-and-innovation/strategy/strategy-2020-2024/democracy-and-rights/gender-equality-research-and-innovation_en
[2] http://genderedinnovations.stanford.edu/
[3] https://op.europa.eu/en/publication-detail/-/publication/d15a85d6-cd2d-4fbc-b998-42e53a73a449

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While WP6 work (D6.6.) is focused on gendered innovations for nanotechnology by providing recommendation for future ERA of ENMs, D5.3 provides the main rationale for including sex/gender as a dimension in all future human hazard assessment of ENMs. It also presents the main results of a meta-analysis performed on the available animal data on nano-specific sex differences to ENMs exposure.

1.1. Target audience

All data, analysis and recommendation have been targeted towards but not limited to the following audience:

- Research scientists (academia to industry)
- Regulatory scientists
- Nanosafety-, nanotoxicology-, genetic toxicology-, human health-related industry sectors which regularly assess ENMs by toxicology-based assays and biological testing
- Governmental bodies
- Non-governmental bodies
- Scientific community outside EU projects (PhD students, Research students)
- Other EU projects and their respective partners
- EU NanoSafety Cluster
- Nanomedicine European Technology Platform (ETPN).

1.2. Summary of progress

The technical and scientific progress made at this stage has primarily been focused towards regulatory and policy guidelines and recommendations related to sex/gender-oriented RA and to explore the impact of sex differences following ENMs exposure. The whole set of data gathered through this meta-analysis is currently being further analysed, across several project partners where each partner is providing feedback on their impact towards development of an improved, robust, predictive and nano-specific framework of regulatory-oriented approaches for human RA. This will allow us to refine and improve the meta-analysis to ensure they are as attractive, effective and supportive as possible. Detailed analysis of data gathered on sex-related response to ENMs under *in vivo* settings will be used for preparation of two Open Access publication, while this Deliverable presents only brief summary of this meta-analysis along with overview of main regulatory and policy guidelines and recommendations for inclusion of sex/gender dimension in research and innovation actions.

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2. Technical & Scientific progress

2.1. Objectives

Main aim of WP5 is to support ENMs Risk Governance by delivering a more efficient and reliable ENM-tailored safety testing strategy, to improve and enhance the tools supporting risk decision making. Following this and based on the initiatives for gender innovations, important objectives of Task 5.3. was to analyse regulatory and policy guidelines and recommendations related to sex/gender-oriented hazard assessment and to explore the impact of sex differences following ENM exposure as an important aspect of regulatory-oriented, science-based human hazard assessment.

2.2. Analysis of regulatory and policy guidelines and recommendations

2.2.1. Definitions

Definitions of sex/gender should be clear, harmonized worldwide and readily available for different stakeholders involved in the R&I sector. Table 1 provides definitions given by different authorities.

Table 1. Sex and gender defined by European, United States (US) and Canadian authorities and agencies.

Authority	Sex definition	Gender definition
European Commission (EC)	Sex refers to biology. In humans, sex refers to the biological attributes that distinguish male, female, and/or intersex. In non-human animals, sex refers to biological attributes that distinguish male, female, and/or hermaphrodite. In engineering & product design research, sex includes anatomical and physiological characteristics that may impact the design of products, systems, and processes.	Gender refers to sociocultural norms, identities, and relations that: 1) structure societies and organizations; and 2) shape behaviors, products, technologies, environments, and knowledges.[4] Gender attitudes and behaviors are complex and change across time and place. Importantly, gender is multidimensional[5] and intersects with other social categories, such as sex, age, socioeconomic status, sexual orientation and ethnicity. Gender is distinct from sex.[6]
US National Institutes of Health (US NIH)	"Sex" refers to biological differences between females and males, including chromosomes,	"Gender" refers to socially constructed and enacted roles and behaviors which occur in a historical and cultural context and vary across

[4] Schiebinger, L. (1999). *Has Feminism Changed Science?* Cambridge: Harvard University Press
[5] Hyde, J. S., Bigler, R. S., Joel, D., Tate, C. C., & van Anders, S. M. (2018). The future of sex and gender in psychology: Five challenges to the gender binary. *American Psychologist*, 74(2), 171-193.
[6] Fausto-Sterling, A. (2012). The Dynamic Development of Gender Variability. *Journal of Homosexuality, 59,* 398-421.

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	sex organs, and endogenous hormonal profiles.	societies and over time. All individuals act in many ways that fulfil the gender expectations of their society. With continuous interaction between sex and gender, health is determined by both biology and the expression of gender.
Canadian Institutes of Health Research (CIHR)	Sex refers to a set of biological attributes in humans and animals. It is primarily associated with physical and physiological features including chromosomes, gene expression, hormone levels and function, and reproductive/sexual anatomy. Sex is usually categorized as female or male but there is variation in the biological attributes that comprise sex and how those attributes are expressed.	Gender refers to the socially constructed roles, behaviours, expressions and identities of girls, women, boys, men, and gender diverse people. It influences how people perceive themselves and each other, how they act and interact, and the distribution of power and resources in society. Gender identity is not confined to a binary (girl/woman, boy/man) nor is it static; it exists along a continuum and can change over time. There is considerable diversity in how individuals and groups understand, experience and express gender through the roles they take on, the expectations placed on them, relations with others and the complex ways that gender is institutionalized in society.

2.2.2. Key Governmental, Agency, and Institutional Policies related to Sex and Gender Research

Development and implementation of sex/gender dimension in the R&I activities started in 1960s with Women's Health Movement in the US and the UK. In the US, Equal Pay Act was established in 1963, the Civil Rights Act in 1964 prohibiting employers and labour unions from discriminating on the basis of sex, the Affirmative Action Applied to Women in 1967, the Equal Employment Opportunity Act in 1972. The regulatory activities of the EU started more than decade later with the European Council Equal Pay Directive in 1975 giving rise to legal, regulatory, and administrative means of ensuring equal pay for equal work and with the European Council Equal Treatment Directive in 1976 to establish the equal treatment of women and men. In the next decades, regulation on sex/gender equity evolved in many sectors, while significant regulatory changes for science, engineering, and technology occurred in 1998 when the Council of Europe defined gender mainstreaming as "the (re)organisation, improvement, development and evaluation of policy processes, so that a gender equality perspective is incorporated in all policies, at all levels and at all stages, by the actors normally involved in policymaking." In the same year, the European Technology Assessment Network (ETAN) was founded as a working group of female scientists for collecting national-level data on women in science, and proposing recommendations to address gender in research and the balancing of scientific careers and family life, while the Advancement of Women and Minorities in Science, Engineering, and Technology

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Development Act was established in the US. From that time, policy and regulatory activities were constantly reinforced worldwide.

As one of the most significant projects launched to provide scientists and engineers with practical methods for sex/gender analysis is the Gendered Innovations project,[2] which involves experts from across the U.S. and the EU 27 Member States. Gendered Innovations was initiated at Stanford University in 2009, while collaborations with the EU started in 2012 after the EC set up an expert group "Innovation through Gender" in 2011. The Gendered Innovations project was presented to the European Parliament on July 9, 2013, which resulted in publication "Gendered Innovations: How Gender Analysis Contributes to Research" with a foreword by European Commissioner Máire Geoghegan-Quinn., Gendered Innovations was the theme of the Gender Summit 6 Asia-Pacific, Seoul, South Korea in August 2015. From 2018-2020, the Horizon 2020 Expert Group, Gendered Innovations (G12), updated and expanded the Gendered Innovations methods and case studies. These activities resulted by the "Gendered Innovations 2: How Inclusive Analysis Contributes to Research and Innovation", published by the Luxembourg: Publications Office of the European Union in 2020 and edited by Londa Schiebinger and Ineke Klinge, with a foreword by European Commissioner for Innovation, Research, Culture, Education and Youth, Mariya Gabriel. In 2021, OECD published "Gender and the Environment: Building Evidence and Policies to Achieve the SDGs".[7]

Best practices in obligatory implementation of sex/gender dimension and analysis for R&I are presented in Table 2.

Table 2. The use and implementation of sex/gender analysis and dimension by different institutions.

Institution	**Document**
European Commission	Horizon Europe Programme Guide (states "The integration of the gender dimension into R&I [research and innovation] content is mandatory. It is a requirement set by default across all Work Programmes, destinations and topics, unless its non-relevance for a specific topic is specified in the topic description, e.g. by the mention 'In this topic the integration of the gender dimension (sex and gender analysis) in research and innovation content is not a mandatory requirement.'"
Canadian Institutes of Health Research	Criteria for Integrating Sex and Gender in Biomedical Research; Criteria for Integrating Sex and Gender in Research with Human Participants
US National Institutes of Health	Include Sex As a Biological Variable (SABV) in all phases of research. If SABV is not included, the applicant must include a "strong justification from the scientific literature, preliminary data, or other relevant considerations."
National Sciences and	Guide for applicants

[7] https://doi.org/10.1787/3d32ca39-en

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Engineering Research Council of Canada	
National Research Foundation, the Republic of Korea (NRF)	Framework Act on Science and Technology

2.3. Main implications of sex differences for human hazard assessment and toxicology

Health hazard assessment aims to estimate the probability of adverse outcomes after exposure to certain chemicals and materials and to recommend acceptable values for such exposure. Owing to sex/gender-related differences in constitutive and physiological parameters, this dimension has to be included also in all approaches and methods for hazard assessment. There are anthropometric (e.g. height, weight, body surface area) and body composition differences (e.g. fat content, muscle mass) between males and females that may affect exposure concentrations of chemicals and materials from different pathways. These differences may also influence the absorption, distribution, metabolism and elimination of agents and have a significant influence on toxicity. Moreover, men and women differ in many lifestyle and occupational exposure factors that may influence the exposure and effect of an agent on the individual. For many chemical toxicants there are important differences between males and females in experimental studies. Despite these differences, sex/gender dimension have been largely neglected by researchers.[8] In a paper by Freeman et al.[9] out of 165 reviewed protocols, only 24 (14.5%) provide reasoning for choice of sex/gender of studied population. In the same paper, the authors concluded that when it comes to topics in which the sex/gender differences might be present, only 2% of papers considered sex or gender effects on the primary outcome. Most of the currently available information on this topic originate from evaluating absorption, distribution, metabolism and excretion (ADME) of drugs, pesticides, biocides and other chemicals,[10] although results from those studies are often referred to a specific agent and do not provide general information on sex differences in ADME processes or their underlying mechanisms. Another problem is that women in general, but mostly women of childbearing age are mainly excluded from many studies.

[8] Weiss B. Same sex, no sex, and unaware sex in neurotoxicology Bernard. Neurotoxicology. 2011;32(5):509–17.
[9] Freeman A, Stanko P, Berkowitz LN, Parnell N, Zuppe A, Bale TL, et al. Inclusion of sex and gender in biomedical research: survey of clinical research proposed at the University of Pennsylvania. Biol Sex Differ [Internet]. 2017 Dec 21;8(1):22. Available from: https://bsd.biomedcentral.com/articles/10.1186/s13293-017-0139-5
[10] Gochfeld M. Framework for gender differences in human and animal toxicology. Environ Res. 2007;104(1):4–21.

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2.3.1. Exposure differences between males and females

Differences in exposure patterns represent the first point to be considered during health hazard assessment. When considering sex/gender dimension, this is reflected by different exposure time for men and women to certain agents as they spent different time in home, community or workplace environment. Type of diet, occupations and lifestyle significantly affect exposure to stressors. Males and females perform different activities and gender-related behaviour is apparent already from the very early stages of infancy. Moreover, disparities between women and men may thus significantly induce sex/gender-related exposure differences.

2.3.2. Sex differences in toxicokinetics and toxicodynamics

The internal dose of certain health stressor depends on toxicokinetics (TK) which can be defined as the study of kinetics of ADME of a xenobiotic under the conditions of toxicity evaluation. The TK is determined biophysical constitution, body composition, physiology and metabolising enzymes. All these parameters differ between males and females. The factors affecting absorption are route specific (oral, dermal, inhalatory) but also sex-specific, e.g. different activity of gastric alcohol dehydrogenase or different transport proteins in kidneys between males and females.

Dermal absorption depends on the condition of skin, hair follicle number, perspiration, skin thickness, presence of adipose tissue (larger in females) and use of cosmetic products.[11] Oral administration implies absorption in the gastrointestinal system and the bioavailability depends on many factors such as gut motility, pH, activity of gastric and intestinal enzymes, intestinal motility, microbiome diversity and expression of transporters.[12,11] In males, the gastric fluid tends to be more acidic and the gastric emptying time is longer, while the transit times is shorter than in females.[12,13] Once absorbed, most agents bind to plasma proteins and their distribution in the body is affected by multiple body composition parameters. Given that total body water volume, blood volume and plasma volume are higher in males, the volume of distribution (Vd) is generally considered to be higher in males, which implies that the concentration of exogenous substances in blood will be lower in males.[14] Another parameter important when considering biodistribution is binding to plasma proteins. Sex-related differences have been found in α1-acid glycoprotein concentrations, which vary dependently on

[11] Arbuckle TE. Are there sex and gender differences in acute exposure to chemicals in the same setting? Environ Res. 2006;101(2):195–204.
[12] Nicolas JM, Espie P, Molimard M. Gender and interindividual variability in pharmacokinetics. Drug Metab Rev. 2009;41(3):408–21.
[13] Soldin OP, Mattison DR. Sex Differences in Pharmacokinetics and Pharmacodynamics. Clin Pharmacokinet. 2009;48(3):143–57.
[14] Soldin OP, Chung SH, Mattison DR. Sex differences in drug disposition. J Biomed Biotechnol. 2011:7–9.

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oestrogen.[14] However, females have larger percentage of body fat, therefore the Vd of lipophilic substances may be higher in females.[12] In toxicology, higher Vd implies lower elimination time, tissue accumulations and possible toxic reactions (e.g. sudden weight loss and release of substance stored in adipose tissue).

Biotransformation or metabolism of toxicants, drugs or other exogenous substances appears to be amongst most significant sex-related variabilities[15] and it is also the most extensively represented in research carried out to this moment. Sex-related differences in metabolism has previously been confirmed in rats with some cytochrome P450 enzyme (phase I reactions) expression descripted as sex-specific. Expression of CYP2C11, CYP2C13 and CYP3A2 is associated with male rats, while CYP2C12 is expressed in female rats.[15] This paper also claims that there are sex-related differences in variety of laboratory model animals as well as humans. Many studies on expression and activity of cytochrome P450 enzymes confirm such findings claiming that CYP3A4 activity is higher in females, while CYP2D6, CYP1A2 and CYP2E1 are more prominent in males.[16] Additionally, activities of enzymes are susceptible to hormonal changes along pregnancy or menopause[17] Phase II enzymes catalyse conjugation reactions which make substances more hydrophilic and therefore more prone to excretion. Variabilities between males and females in humans have been noticed in sulfotranferases, methyl-tranferases and UDP-glucuronosyl-transferases.[13,14] It is also worth mentioning that males have higher basal metabolic rates which can be attributed to males having higher percentage of muscle tissue compared to females who have higher percentage of adipose tissue.

Elimination is susceptible to sex related differences as well. Exogenous substances are eliminated from organism through different routes: urine, feces, lungs, skin then exit the organism, latter of which is specific for women. As for renal elimination, some studies show that females have lower renal blood flow which results in lower GFR (glomerular filtration rate) and can lead to longer retention of some substances in females because renal clearance (Cl) is lower. Another factor that causes dissimilarities in male and female renal excretion is the difference in expression of transporters present in kidneys.[18,19] As an example, perfluorooctanoic acid (PFOA) has been shown to is transported via organic anion transporters (OATs) present in proximal renal tubule cells. Studies show that the expression OATs is sex-specific and results in faster elimination of PFOA in female versus

[15] Czerniak R. Gender-based differences in pharmacokinetics in laboratory animal models. Int J Toxicol. 2001;20(3):161–3.
[16] Wolbold R, Klein K, Burk O, Nüssler AK, Neuhaus P, Eichelbaum M, et al. Sex is a major determinant of CYP3A4 expression in human liver. Hepatology. 2003;38(4):978–8
[17] Scandlyn MJ, Stuart EC, Rosengren RJ. Sex-specific differences in CYP450 isoforms in humans. Expert Opin Drug Metab Toxicol. 2008;4(4):413–24.
[18] Joseph S, Nicolson TJ, Hammons G, Word B, Green-Knox B, Lyn-Cook B. Expression of drug transporters in human kidney: Impact of sex, age, and ethnicity. Biol Sex Differ. 2015;6(1):1–15.
[19] Trevisan A, Chiara F, Mongillo M, Quintieri L, Cristofori P. Sex-related differences in renal toxicodynamics in rodents. Expert Opin Drug Metab Toxicol. 2012;8(9):1173–88.

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male rats.[20] Toxicodynamics (TD) describes the dynamic interactions between a compound and its biological target, leading ultimately to an (adverse) effect. A biological target, also known as the site of action, can be binding proteins, ion channels, DNA, or a variety of other receptors. The most significant TD difference between the two sexes is QT interval change (the section on the electrocardiogram (ECG) that represents the time it takes for the electrical system to fire an impulse through the ventricles and then recharge) and females tend to be more susceptive to it because testosterone has beneficial effects on prolonged QT interval syndrome.[21,22] Another TD aspect that is affected by sex hormones, primarily oestrogen, is nociception. Females seem to be less responsive to pain medications than males.[23]

In general, main TK factors causing women more sensitive to certain stressor is the smaller volume of distribution in women compared to men, larger free fraction of agent in the circulation and slower clearance from the body. With regard to the TD, women are more sensitive due to the alteration in receptor number and binding, as well as in signal transduction pathway following receptor binding.[13]

Main physiological conditions that affect sex difference in TK/TD are pregnancy and menopause. While data TK changes during menopause are still conflicting, physiologic changes during pregnancy are known to affect TK of xenobiotics including volume of distribution, increased plasma volume, extracellular fluid space and total body water, regional blood flow changes (increased uterine, renal, skin and mammary blood flow, decreased skeletal blood flow), increased stroke volume (early pregnancy), increased heart rate (later in pregnancy), respiratory changes (compensated respiratory alkalosis, pH-7.44), decreased plasma albumin, absorption changes such as prolonged gastric evacuation time, liver CYP450 enzyme and uridine diphosphate glucoronosyltransferase (UGT) isoenzyme changes, increased renal blood flow.[13] Therefore, health hazard assessment of pregnancy requires special attention.

[20] Kudo N, Katakura M, Sato Y, Kawashima Y. Sex hormone-regulated renal transport of perfluorooctanoic acid. Chem Biol Interact. 2002;139(3):301–16.
[21] James AF, Hancox JC. Sex, drugs and arrhythmia: Are gender differences in risk of torsades de pointes simply a matter of testosterone? Cardiovasc Res. 2003;57(1):1–4.
[22] Burke JH, Ehlert FA, Kruse JT, Parker MA, Goldberger JJ, Kadish AH. Gender-specific differences in the QT interval and the effect of autonomic tone and menstrual cycle in healthy adults. Am J Cardiol. 1997;79(2):178–81.
[23] Farkouh A, Riedl T, Gottardi R, Czejka M, Kautzky-Willer A. Sex-Related Differences in Pharmacokinetics and Pharmacodynamics of Frequently Prescribed Drugs: A Review of the Literature. Adv Ther. 2020;37(2):644–55.

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2.4. Meta-analysis of nano-specific sex differences

2.4.1. Methodology

The systematic literature review on sex-related differences for response to ENMs exposure under in vivo settings was carried out this in the *PubMed* and Web of Science databases using keywords as presented in Table 3. The last search was done on November 10th, 2021. Final selection was made for the articles searched by keywords "(("rat") OR ("mice") OR ("animal")) AND ("nano*") AND ("sex")" which gained 667 articles from the PubMed and 657 articles from the Web of Science database. These articles were then checked for quality by using approach developed under GUIDEnano project[24] (Fernández-Cruz et al., 2018) for quantitative evaluation of the quality of environmental and human toxicity studies performed with ENMs. This approach is based on the use of K and S scores. K score is related to test design and reporting considerations following the principles of the ToxRTool,[25] while S score is based on the physicochemical properties that have been characterized and reported for the NMs including properties characterized in the exposure medium. However, due to our specific aim to extract data relevant for targeting sex analysis in response to ENMs, we have modified above mentioned approach by adding extra questions as presented in Tables 4 and 5.

Table 3. Number of papers (No.) found during literature research sorted by database and searched keywords. If the papers from database search using certain keywords were included in further analysis, number of papers is marked with *.

Keywords	No. PubMed	No. Web of Science
(("in vivo") AND ("nano*"))	58256	97520
(("in vivo") AND ("nano*")) AND ("sex")	124*	163*
(("in vivo") AND ("nano*")) AND ("gender")	36*	69*
(("rat") OR ("mice") OR ("animal")) AND ("nano*")	146801	81362
(("rat") OR ("mice") OR ("animal")) AND ("nano*") AND (exposure"))	11025	7926
(("in vivo") OR ("animal")) AND ("exposure") AND ("nano*")	11542	6765
(("rat") OR ("mice") OR ("animal")) AND ("nano*") AND ("sex")	667*	657*
(("rat") OR ("mice") OR ("animal")) AND ("nano*") AND (exposure") AND ("sex")	144*	153*
(("in vivo") OR ("animal")) AND ("exposure") AND ("nano*") AND ("sex")	139*	96*

[24] Fernández-Cruz M.L. et al. Environ. Sci.: Nano, 2018,5, 381-397
[25] https://publications.jrc.ec.europa.eu/repository/handle/JRC51252

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Table 4. K-score used generally to analyse quality of papers are shown in left column. In the right column are questions used to assess K-score adapted for this literature research. Questions marked with * had to be answered positively.

Questions from GuideNano	**Questions used in this literature research**
Is the test model given?*	Is the test model given and appropriately described?*
Is information given on the source/origin of the cell line?	Is information given on the source/origin of the animal model?
Are necessary information on test system properties, and on conditions of cultivation and maintenance given?	Are necessary information on test system properties, and on conditions of cultivation and maintenance given?
Is the method of administration given?*	Is the method of administration given?*
Are duration of exposure as well as time-points of observations explained?*	Are duration of exposure as well as time-points of observations explained?*
Were negative and positive controls included (where and when needed)?*	Were negative and positive controls included (where and when needed)?*
Is the number of replicates (or complete repetitions of experiment) given?*	Is the number of replicates (or complete repetitions of experiment) given?*
Are the study endpoint(s) and their method(s) of determination clearly described?*	Are the study endpoint(s) and their method(s) of determination clearly described?*
Have the results been analysed using statistical methods?*	Have the results been analysed using statistical methods?*
-	Were both female and male animals used?*
-	Were results from females and males analysed separately?*
-	Were differences between females and males statistically analysed?

Table 5. S-score to analyse quality of papers according to the approach of the GuideNano project and used in this work. Questions marked with * had to be answered positively.

S-score questions from GuideNano
Was the test ENM identified?*
Is information on the source of the ENM given?*
Is purity (concentration) of the ENM given?*
Is endotoxin content of the ENM given?

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Were impurities stated?
Was the type of test medium or vehicle used stated?*
Were protocols of dispersion and characterization in the exposure medium identified? or, were protocols of preparation of exposure medium stated?
Was the ENM concentration measured in the exposure medium?
Was the stability of the ENM concentration measured during the exposure period?
Are doses administered or concentrations in exposure media given?*
Primary particle Size*
Particle Size in media*
Size at the start or at the end of the exposure period*
Surface area
Surface charge
Surface Charge in media
Shape*
Other relevant information (i.e. crystal structure, solubility, magnetic properties, acidity/basicity, redox potential, catalysis, photosensitivity, hydrophobicity, radical production capacity, etc.)

2.4.2. Main results

The quality scoring of all papers retrieved by the search in the PubMed and Web of Science databases using the GuideNano approach finally resulted in 171 papers according to the K-score (as presented in Figure 1).

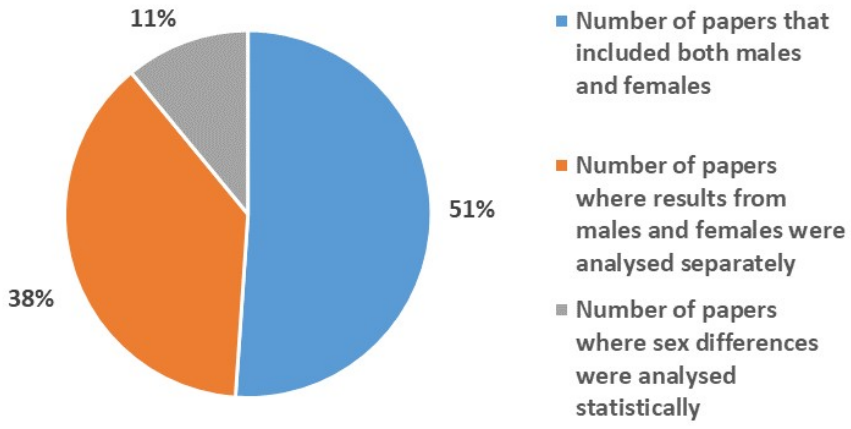

Figure 1. Analysis of 171 papers chosen from literature research for detailed analysis of K-score and S-score.

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Although research studies presented in these 171 papers included animals of both sexes, only 69 studies showed results separately for males and females, while only 20 out of these 69 studies statistically analysed sex-related differences. This finding was quite disappointing considering all policy and regulatory requirements about implementing sex/gender dimension and analysis in the R&I activities (as presented in section 2.2). Papers that were selected by K-score were further refined by the existence of data for response of males and females separately, which resulted in 69 papers. These were further analysed by S-score quality criteria which resulted in 51 papers (presented in Table 6). Summary of data showed in these papers separately for male and female animals regarding tested ENMs type, animal model used, exposure period and tested ENMs doses is presented in Table 6. Summary of main findings on the toxicity endpoints that were affected by ENMs during *in vivo* testing are presented in Table 7.

Table 6. The list of papers that have reported response to ENMs exposure of males and females separately.

DOI	ENM core	Shape	Surface functionalization	Primary size	Hydrodinamic diameter	Animal model	Exposure	Dose
10.1002/jat.2742	Ag	spherical	None	10-30 nm, average 21.8 nm	3-117 nm, average:90.5 nm	ICR mice	7 days and 14 days	7.5, 30.0 and 120.0 mg/kg b.w.
10.1093/toxsci/kfv318	Ag	spherical	citrate	10, 75, and 110 nm	17.43 ± 0.07, 74.35 ± 0.62 and 104.3 ± 12.75	Sprague Dawley/CD-23 rats	13 weeks	9, 18, and 36 mg/kg bw
10.1016/j.impact.2020.100221	Ag	spherical	None	20 nm	20 nm	Sprague Dawley/CD-23 rats	3 days	50, 150 and 300 mg/kg bw
10.1080/15287390903212287	Ag	spherical	CMC	60 nm	-	CD1 mice	90 days	30, 125 and 300 mg/kg
10.3390/ijms22010009	Ag	spherical	citrate	10, 20, 75 and 110 nm	-	Fischer 344 (F344) rats	24 hours	20 µg/mL
10.2131/jts.40.263	Ag	spherical	citrate	32 +/- 6.6 nm	-	human terminal ileum	From day 3 of gestation every 3 days until	0.2 and 2 mg/kg

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						tissue	parturition	
10.1002/ppsc.201900174	Ag	spherical	PVP, albumin, metallothionein	10 nm	PVP: 8.6 ± 1.9 nm, ALB: 14.9 ± 2.8 and 37.7 ± 6.5 nm and MT: 7.6 ± 0.7 and 13.9 ± 11.3 nm	NMRI mice	1 hour	1 mg of Ag/kg bw
10.1186/s12989-021-00425-y	Ag	spherical	PVP	8.6 ± 1.9 nm	12.1 ± 3.4 nm	Wistar rats	28 days	0.1 and 1 mg/kg
10.2147/IJN.S46376	Ag	spherical	Citrate	4.4, 22.5, 29.3, and 36.1 nm	-	Wistar rats	28 days	4000 µg/kg
10.1016/j.impact.2021.100340	Ag	Spherical	TRF and PVP	PVP: 12.2 +/- 4.2 nm and TRF: 11.3 +/- 4.6 nm	PVP: 14.7 +/- 12.2 nm and TRF: 50.8 +/- 6.8 nm	C57 mice	21 days	1 mg Ag/kg b.w
10.1016/j.impact.2020.100255	Ag	Spherical	CMC	52.7–70.9 nm	56 nm and 1.46	C57Bl/6 Albino	28 days	500 mg/kg
10.1080/08958370701874663	Ag	spherical	CMC	60 nm	-	Sprague Dawley rats	28 days	30 mg/kg/day
10.1039/C8RA00044A	Ag, Au	Ag core/Au shell nanorods	None	average length: 73.4 ± 3.2 nm, average diameter: 34.6 ± 1.9 nm	not defined	Sprague Dawley rats	1 i.v. dose	300 and 1000 mg/kg/day
10.1016/j.taap.2020.114890	Amorphous silica	spherical	None	20 nm and 50 nm	around 20 and 50 nm	Sprague Dawley rats	2 weeks, 3 times per week	injected volume per rat was 500 mL, containing 0.6 mg Ag and 0.34 mg Au.
10.1016/j.nano.2014.10.005	Au	The nanoparticles typically had 2-9 branches with average tip-to-tip distances ca. 50 nm	None	ca. 50 nm	68.8 ± 1.8 nm	Sprague Dawley rats	i.v. bolus injection, single dose	12.5, 25, or 50 µg of AuSiNPs
10.1186/1743-8977-	Au	spherical	None	4-5 nm	-	Sprague–Dawley	6 h/day, 5 days/week,	Apt-AuNS at 0.48. 4.8, 9.6

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8-16						(Crl:SD)	for 13-weeks	and 48 mg/kg b.w.
10.3109/17435390.2014.933903	Carbon	Nanotubes	None	width: 90.7 nm; length: 5.7 mm	-	Sprague Dawley rats	13 weeks	2.5×10^4, 2.5×10^5, and $1.2\text{-}2.8 \times 10^6$ particles/mL
10.1093/toxsci/kfs172	Carbon	Nanofiber	None	5.8 μm in length (range of 1–14 μm) and 158 nm in diameter	-	F344/DuCrlCrlj rats	90 days	0.2, 1 and 5 mg/m^3
10.1093/toxsci/kfs172	Carbon	nanotubes	None	2 nm and 30 nm (diameter)	-	Crl:CD Sprague Dawley rats	28 days	0.50, 2.5, or 25 mg/m^3
10.1080/08958378.2019.1669743	Carbon	nanotubes	none	27 nm in diameter, 5–15 μm in length	-	Crl:CD Sprague Dawley rats	24 hours, 7 days and 12 weeks	SWCNT: 0.125, 1.25, 12.5 mg/kg/day and MWCNT: 0.5, 5 and 50 mg/kg/day
10.1080/17435390.2016.1202348	Carbon	nanotubes	None	100–1000 nm	89.9±11.9 and 181.1±29.8 nm	C57BL/6 mice	90 days	1 mg/ml
10.1007/s00210-020-01899-x	Copper ferrite	tetragonal crystals	None	14.06 nm	-	ICR mice	14 days	100 and 50 μg/kg
10.2147/IJN.S106346	Cu	spherical	HPMC	32.7±10.45 nm	516.4±116.9 nm	Wistar rats	14 days	10 mg/mL saline/bw
10.1016/j.envres.2016.08.025	Fe	rods	None	-	209.4 ± 98	Sprague-Dawley rats	Before pregnancy	312, 625, 1250, and 2500 mg/kg
10.1293/tox.2013-0036	Fe_3O_4	Spherical	none	5-15 nm	-	ICR mice	The rats were given a total of 13 quadweekly intermittent exposures during the experimental period of 52 weeks	1, 2 and 4 mg/kg
10.1038/srep35053	inorganic matrix of polysiloxane	not specified	None	-	3.5 ± 1 nm	Fischer 344 (F344/DuCrlCrlj)	7 days	0.2, 1 and 5 mg/kg

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						rats		
10.1007/s11011-018-0248-9	La-Zirconate	Characterized in another paper	Characterized in another paper	Characterized in another paper	Characterized in another paper	cynomolgus monkeys (macaca fasciculari s)	22 days	150, 300, and 450 mg/kg/administration
10.1007/s00210-020-01819-z	lanthanum titanate	spherical	None	14 to 42 nm	-	albino mice	15 days and 29 days	75 mg/ml solvent/kg b.w.
10.1002/jat.2887	MnO_2	irregural spherical	None	42.63 +/- 23nm	-	albino mice (C57BL/6 strain)	28-day repeated dose oral toxicity study	50 mg/ml saline/kg b.w.
10.1186/s12989-016-0164-2	Multi-walled carbon nanotubes	nanotubes	None	40-90 nm	-	Wistar rats	exposed to MWNT-7 aerosol for 104 weeks (6 h/day, 5 days/week)	30, 300 and 1000 mg/kg b.w./day
10.1038/s41398-020-00907-1	nanosized particulate matter from polluted air	not defined	None	0.2 µm	not defined	Fischer 344 (F344/DuCrlCrlj) rats	for 3 weeks, 3 days per week, 5 hours a day	0, 0.02, 0.2, or 2 mg/m^3
10.1080/17435390.2020.1808105	Ni	spherical	None	20 nm	25.43 ±11.62	C57BL/6J mice	24 days	300 µg/m^3
10.1080/1354750X.2020.1841829	NiO	spherical	None	40-60 nm	not specified	C57BL/6J mice	14 days	4 mg/kg
10.1016/j.toxlet.2020.01.008	polyethylene	irregural spherical	acid and hydroxy groups	-	16.9 ± 1.9 µm	albino mice (C57BL/6 strain)	90 days	21 mg/ml saline/kg bw and 50 mg/ml saline/kg bw
10.2147/IJN.S57939	Si	spherical	None	15±3 nm and 89±14 nm	-	ICR mice	Single oral gavage	0.125, 0.5, 2 mg/day/mouse
10.2147/IJN.S57925	Si	spherical	None	20 nm and 100 nm	21.0±0.1 nm and 91.6±0.5 nm	Sprague Dawley rats	15 days	500 or 1,000 mg/kg
10.1016/j.jconrel.2020.05.027	Si	-	None	432 ± 18.7 nm and 46 ± 4.9 nm	-	Crl:CD(SD) rats	1 year	500, 1000, 2000 mg/kg
10.1016/j.fct.2020.1	SiO_2	spherical	None	13-45 nm	53.9-195.9 nm	Balb/C	90 days	100 mg/kg

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11168		amorphous				mice		
10.3390/molecules18077460	Thymoquinone	nanoemulsion	None	-	-	Sprague-Dawley rats	2 weeks	2, 5, 10, 20 and 50 mg/kg b.w./day
10.1002/jat.3985	TiO_2	spherical anatase crystals	None	24 ± 5 nm	40.8 ± 0.38 nm	Sprague Dawley rats	90 consecutive days	20 mL/kg b.w.
10.1002/jat.3769	TiO_2	nearly spherical and anatase crystals	none	24 ± 5 nm	around 50 nm	Sprague-Dawley rats	90 days	TiO_2 NPs (0, 2, 10 and 50 mg/kg BW), glucose (1.8 g/kg BW) and TiO2 NPs (0, 2, 10 and 50 mg/kg BW) + glucose (1.8 g/kg BW)
10.3109/17435390.2013.822114	TiO_2	spherical and irregular	None	spherical: 20-60 nm; irregular: 40-60 nm	284 +/- 43 nm	Sprague Dawley rats	5 days	0, 2, 10 and 50 mg/kg bw
10.1016/j.fct.2017.01.031	TiO_2	Spherical and irregular	None	25 nm	604 ± 24	Sprague Dawley rats	5 days	1 and 2 mg/kg/day
10.5625/lar.2015.31.3.139	ZnO	spherical	L-Serine	100 nm	not specified	Sprague Dawley rats	14 days	2 or 1 mg/kg bw per day
10.1039/c3nr02140h	ZnO	Spherical	L-Serine and citrate	20 and 70 nm	around 20 and 70 nm	Sprague Dawley rats	7 days	500, 1000 and 2000 mg/kg bw/day
10.1002/jat.2862	ZnO	large agglomerates	None	40 nm	201.75 +/- 17.15 nm	Sprague Dawley rats	13 weeks (90 days)	50, 300 and 2000 mg/kg
10.2147/IJN.S57928	ZnO	spherical	L-Serine and citrate	100 nm	-	Sprague Dawley rats	90 days	67.1, 134.2, 268.4 or 536.8 mg/kg bw/day
10.2147/IJN.S57927	ZnO	not defined	L-Serine	20 nm	29+/-3 nm	Sprague Dawley rats	90 days treatment and 14 days recovery	31.25, 125, 500 mg/kg

Table 7. Main toxicity endpoints that were affected in animal models exposed to different ENMs types, with specified ENMs that caused sex-related response. Data were extracted from 51 papers presented in Table 6.

Toxicity endpoint	**Affecting ENMs**	**ENMs caused sex-**

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		related response
Food intake	SWCNT, MWCNT, thymoquinone, PE-MPs, ZnO, TiO_2, SiO_2, Fe_3O_4, amorphous silica, Si, Au, Ag	ZnO, SiO_2, Fe_3O_4, amorphous silica, Si
Water consumption	thymoquinone, PE-MPs, ZnO, TiO_2, amorphous silica, Si, Ag	ZnO, Si
Weight gain	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, TiO_2, SiO_2, MnO_2, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag, LTNPs	Thymoquinone-NPs, PE-MPs, ZnO, SiO_2, Au
Organ weight - heart	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, TiO_2, Fe_3O_4, amorphous silica, Si, Au, Ag	ZnO
Organ weight - brain	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, TiO_2, SiO_2, Fe_3O_4, Si, Au, Ag	Ag
Organ weight - liver	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, TiO_2, SiO_2, Fe_3O_4, amorphous silica, Si, Au, Ag	SiO_2, Ag
Organ weight - kidney	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, TiO_2, SiO_2, Fe_3O_4, amorphous silica, Si, Au, Ag	ZnO
Organ weight - adrenal gland	SWCNT, MWCNT, ZnO, TiO_2, SiO_2, Fe_3O_4, amorphous silica, Si, Au, Ag	ZnO
Haemoglobin	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag, LTNPs	PE-MPs, Ag, LTNPs
Haematocrit	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag, LTNPs	PE-MPs, ZnO, Fe_3O_4, amorphous silica, Ag
MCV	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag, LTNPs	PE-MPs, ZnO, amorphous silica, Ag
MCH	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag, LTNPs	Ag
MCHC	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag, LTNPs	PE-MPs, ZnO, Fe_3O_4, amorphous silica, Ag
Retikulocytes	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag	PE-MPs, ZnO, amorphous silica
Platelet count	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, SiO_2, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag, LTNPs	SWCNT, PE-MPs, SiO_2, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Ag

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Leukocyte	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, Si, Au, Ag	ZnO
Neutrophiles	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag	SWCNT, PE-MPs, ZnO, amorphous silica
Lymphocytes	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, TiO_2, SiO_2, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag, LTNPs	SWCNT, PE-MPs, ZnO, TiO_2, SiO_2, $La_2Zr_2O_7$, amorphous silica, Si
Eosinophiles	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag	SWCNT, PE-MPs, ZnO, amorphous silica
Monocytes	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, TiO_2, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag, LTNPs	SWCNT, PE-MPs, TiO_2, Fe_3O_4, amorphous silica, LTNPs
Basophiles	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag	SWCNT, PE-MPs, ZnO, Fe_3O_4, amorphous silica
White blood cells	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, TiO_2, SiO_2, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag, LTNPs	PE-MPs, SiO_2, $La_2Zr_2O_7$, Fe_3O_4, Ag, LTNPs
Red blood cells	SWCNT, MWCNT, Thymoquinone-NPs, PE-MPs, ZnO, SiO_2, NiO, $La_2Zr_2O_7$, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag	SWCNT, ZnO, SiO_2, Fe_3O_4, Ag
Albumin concentration	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, TiO_2, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Cu, Au, Ag	ZnO, TiO_2, NiO, Ag
Glucose level	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag	ZnO, Fe_3O_4, amorphous silica
Blood urea/nitrogen level	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Cu, Au, Ag	ZnO, NiO, amorphous silica, Cu
Creatinine level	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, SiO_2, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Cu, Au, Ag	ZnO, amorphous silica, A
Total protein content	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, SiO_2, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Ni, Cu, Au, A	ZnO, SiO_2, NiO, Fe_3O_4, amorphous silica, Ni, Cu, Ag
Creatine kinase activity	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Cu, Au, Ag	ZnO, Fe_3O_4, amorphous silica, Si, Cu

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Aspartate aminotransferase activity	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, SiO_2, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Cu, Au, Ag	Thymoquinone-NPs, ZnO, Fe_3O_4, amorphous silica, Si, Ag
Alanine aminotransferase activity	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, SiO_2, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Cu, Au, Ag	SWCNT, ZnO, amorphous silica, Ag
Bilirubine level	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Cu, Au, Ag	ZnO, amorphous silica, Ag
□-glutamyltransferase activity	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag	ZnO, Fe_3O_4, amorphous silica
Alanine phosphatase activity	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Cu, Au, Ag	SWCNT, ZnO, amorphous silica, Ag
Choleseterol level	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Cu, Au, Ag	ZnO, amorphous silica, Cu, Ag
Triglycerides	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Cu, Au, Ag, LTNPs	SWCNT, ZnO, $CuFe_2O_4$, amorphous silica, Cu
Phospholipase activity	SWCNT, MWCNT, Thymoquinone-NPs, NiO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag, LTNPs	amorphous silica, LTNPs
Calcium concentration	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Au, Ag	Fe_3O_4, amorphous silica
Potassium concentration	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Cu, Au, Ag	amorphous silica
Sodium concentration	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Cu, Au, Ag	ZnO, Fe_3O_4, amorphous silica, Cu
Chloride concentration	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, Fe_3O_4, $CuFe_2O_4$, amorphous silica, Si, Cu, Au, Ag	ZnO, Fe_3O_4, amorphous silica, Cu
Inorganic phosphorus	SWCNT, MWCNT, Thymoquinone-NPs, ZnO, $CuFe_2O_4$, amorphous silica, Si, Au, Ag	ZnO, amorphous silica
Accumulation - blood	ZnO, MnO_2, Si, Au, Ag	ZnO, Au, Ag
Accumulation - adrenal gland	Si, Au	-

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Accumulation - liver	ZnO, SiO_2, MnO_2, $CuFe_2O_4$, Si, Au, Ag	SiO_2, Ag
Accumulation - kidney	ZnO, MnO_2, Si, Ag	Ag
Accumulation - spleen	ZnO, SiO_2, MnO_2, Si, Au, Ag	SiO_2, Au, Ag
Accumulation - testis	ZnO, Si	-
Accumulation - ovary	ZnO, $CuFe_2O_4$, Si	-
Accumulation - brain	MnO_2, Si, Ag	-
Accumulation - stomach	ZnO, SiO_2, Si, Ag	ZnO, Ag
Accumulation - intestine	ZnO, SiO_2, Si, Ag	Ag
Accumulation - lung	MWCNT, ZnO, MnO_2, Si, Ag	Ag
Excretion - feces	ZnO, Si, Ag	-
Excretion - urine	MnO_2, Si, Ag	-
Spatial cognition abilities	$La_2Zr_2O_7$	-
HbA1c	TiO_2, $CuFe_2O_4$	TiO_2
Glycated serum protein	TiO_2	TiO_2
Insulin	TiO_2	TiO_2
Glucagon	TiO_2	-
C-peptide	TiO_2	TiO_2
Superoxide dismutase activity	TiO_2, NiO, $La_2Zr_2O_7$, Ag	TiO_2, NiO, $La_2Zr_2O_7$, Ag
Catalase activity	NiO, $La_2Zr_2O_7$, Ag	NiO, $La_2Zr_2O_7$
Glutathione level in blood, liver and kidneys	TiO_2, Ag	TiO_2, Ag
Superoxide radical level in blood, liver and kidneys	Ag	Ag
Glutathione peroxidase activity	Ag	Ag
Peroxy radical level in blood, liver and kidneys	Ag	Ag
Inflammatory response	SWCNT, MWCNT, TiO_2, Fe_3O_4, Ni, Ag	TiO_2, Fe_3O_4, Ag
T3 level	TiO_2, SiO_2	TiO_2, SiO_2
TSH level	SiO_2	SiO_2
DNA damage - blood	MnO_2, Ag	Ag

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DNA damage - liver	Ag	Ag
DNA damage - kidney	Ag	Ag

Detailed analysis of data gathered through described literature search will be presented in two review papers that are under preparation.

2.5. Recommendations for implementing sex dimension and analysis in future R&I activities for nanotechnology

Sex/gender mainstreaming was strictly implemented in European policy since 1998 (see section 2.2). In 1999, the Committee on Understanding the Biology of Sex and Gender Differences was formed by the nonprofit, non-governmental organization National Academy of Medicine (NAM), formerly called the Institute of Medicine (IoM) until 2015. Main goal was to evaluate and provide recommendations on understanding of sex differences and determinants at the biological level, which are summarized in Table 8.[26]

Table 8. Main finding and recommendation of the Committee on Understanding the Biology of Sex and Gender Differences.[26]

Main finding	Recommendation	What should be studied?
Every cell has a sex	Research on sex at the cellular level should be promoted	Determine the functions and effects of X-chromosome- and Y-chromosome- linked genes in cells. Determine how genetic sex differences affect other levels of biological organization, including susceptibility to disease. Identify and distinguish between the effects of genes and the effects of hormones.
Sex begins in the womb	Sex differences should be studied from womb to tomb	Inclusion of sex as a variable in research designs. Reveal the mechanisms of intrauterine effects. Determine how sex differences influence health, illness, and longevity.
	Cross-species information should be mined	Select models that mirror human sex differences and human conditions. Develop appropriate animal models, including those

[26] Institute of Medicine (US) Committee on Understanding the Biology of Sex and Gender Differences. Exploring the Biological Contributions to Human Health: Does Sex Matter? Wizemann TM, Pardue ML, editors. Washington (DC): National Academies Press (US); 2001. PMID: 25057540.

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		involving nonhuman primates. Alert for unexpected phenotypic sex differences resulting from the production of genetically modified animals.
Sex affects behavior and perception	Investigate natural variations	Examine genetic variability, disorders of sex differentiation, reproductive status, and environmental influences
	Research on sex differences in brain organization and function should be expanded	Evaluate sex-differential environmental and behavioral influences on brain organization and function to recognize brain modulators
Sex affects health	Sex differences and similarities for all human diseases should be monitored	Consider sex as a biological variable in all biomedical and health-related research. Design studies to control exposure, susceptibility, metabolism, physiology, and immune response variables, to consider ethical concerns (e.g., risk of fetal injury) constrain, and to detect sex differences across the life span
Challenges and opportunities for inclusion of sex/gender dimension in the R&I	Use of the terms sex and gender should be clarified	Use the term sex as a classification, generally as male or female, according to the reproductive organs and functions that derive from the chromosomal complement. Use the term gender to refer to a person's self-representation as male or female, or how that person is responded to by social institutions on the basis of the individual's gender presentation. Use the term sex in most studies of nonhuman animals .
	All research sponsors should encourage research initiatives on sex differences	Support and conduct additional research on sex differences.
	Sex-specific data should be made more readily available.	Include descriptions of the sex ratios of the research population and specify the extent to which analyses of the data by sex were included in the study.
	Sex of origin of biological research materials should be determined and disclosed.	State, if known, the origin and sex chromosome constitutions of cells or tissue cultures used for cell biological, molecular biological, or biochemical experiments.
	Endocrine status of research subjects should be identified	Facilitate and foster synergies between and among basic scientists, epidemiologists, social scientists, and clinical researchers.

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	Interdisciplinary research on sex differences should be encouraged and supported	Enhance collaboration across medical specialties. Fund better translational—or bench-to-bedside—research and interlevel integration of data (cellular, to animal, to human).
	Potential for discrimination based on identified sex differences should be reduced	Consider historical practices so that they will not be repeated.

3. Deviations from description of action

No major deviation to report until now.

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4. Conclusions

This deliverable describes the analysis of regulatory and policy context of the implementation of the sex/gender dimension and analysis within the R&I activities, as well as the existing animal data that may be relevant for the evaluation of sex-related differences in human response to ENM exposure. The analysis showed of the scarcity of data relevant for proper hazard identification, even when animals of both sexes were included in the study. This is an important indicator for all stakeholders tackled by the field of nanotoxicology and nanosafety to immediately start to properly follow regulatory and policy requests and recommendations on implementation of the sex/gender dimension into their analysis. One of the comfortable pathways to proceed in this direction is to follow the guidelines and decision trees provided by the Gender Innovations project.[2,27]

[27] Tannenbaum C. et al. Nature, 2019, 575, 137-146.

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