D5.1 Report on the state of the art and modeling plan

Revision and history chart

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Abstract

Deliverable 5.1 – the report on the state of the art and modeling plan – serves to summarize the current situation regarding the experimental and computational methods and to define the initial modelling plan that will guide the WP5 along the CheMatSustain project. This includes, a review and discussion of the overall state of the art regarding the assessment of the safety and biodegradability of chemicals and materials, including both the experimental methods (which should provide data for modelling) and the computational methods (which reflect the current technology regarding modelling). Furthermore, it presents the initial steps of data mining and discusses the availability of data and its impact. Finally, the deliverable presents an initial modelling plan that will guide the WP5 by establishing which parameters are going to be prioritized both for tasks T5.2 and T5.3.

Keywords

Computational methods, toxicity, *in silico* methods, *in vitro* methods, data mining.

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Acknowledgement

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List of Abbreviations and Acronyms

ABBREVIATION	FULL NAME
CMS	CheMatSustain
CNMS	Chemicals and (nano)materials
DNA	Deoxyribonucleic acid
MOX	Metal oxide
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MWCNT	Multi-walled carbon nanotube
NAM	New-Approach Method
NANO-QSAR	Quantitative Structure-Activity Relationship for nanomaterials
NM	Nanomaterial
ROS	Reactive Oxygen Species
QSAR	Quantitative Structure-Activity Relationship
XTT	2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide

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Introduction

On the contents and scope of this deliverable

WP5 aim is to explore the use of *in silico* data-based models to identify, group and assess the (eco)toxicity of chemicals and (nano)materials (CNMs). Thus, a series of computational prediction models will be assessed and new models will be developed for a series of chemicals (Task 5.2) and materials (Task 5.3). Finally, some of the most promising models will be implemented in a computational tool to facilitate their application (Task 5.4). However, such advancements require a review of the existing modeling approaches, as well as knowledge on the availability of experimental data to develop the models. Hence, the first phase of the WP5 consists in reviewing the existing methods (computational and experimental) and compiling data related to the projected model (Task 5.1). This report presents a summary of the findings obtained during this phase, as well as a discussion of the consequences of this research in the planning of the following tasks.

Computational models, and in particular QSAR models, for organic, molecular chemicals are a mature technology widely accepted by scientists and administration. However, the development of models for materials is a more challenging issue, due both to the availability of data and additional technical issues. Thus, this deliverable mainly focuses on the models for materials which are the major challenge of the WP5.

Bibliographic research, in addition to providing an overview of the state of the art, is a key component in the preparation of the modelling plan for the WP5. The selection of the endpoints is based both on the relevance for the CMS project (i.e. interaction with other WPs and significance on the SSbD approach) and on the availability of data. An endpoint in this context refers to the exact parameters predicted by the *in silico* models, such as a physicochemical property, certain toxicological or biological activity, or the result of a particular experimental test. Hence, this deliverable includes a description of the data-mining process and a summary of the results. There are plenty of standardized experimental methods for molecular chemicals, (harmonized and accepted for regulatory and scientific objectives), but their application to materials, and in particular to nanomaterials, is not fully standardized and the availability of data is scarcer.

State of the art

Summary of the state of the art in in silico *modelling of chemicals and materials*

A thorough review of the literature has been done regarding the methodology done to assess the toxicity of CNMs. Due to the objectives of the WP5, the main focus was computational modeling, but also experimental methods have been considered (including those methods that are present in the analysis of the data availability).

Toxicity assessment methods of CNMs

The general aim of toxicology is to understand, evaluate and quantify the damage of chemical substances to humans. Hence, clinical data from humans is very informative and can be used to identify toxins. However, it cannot be used systematically to explore new scenarios and experimental toxicology mostly uses data obtained from different models. Toxicological tests are then often classified according to the kind in model as *in vivo, in vitro,* and *in chemico* (in addition of *in silico* that we will discuss separately), depending on the nature of the biological target used to model humans.

There is a significant story of development and enhancement of experimental methods to assess toxicity, both for scientific objective such as the understanding of toxicological mechanisms and as a part of the risk evaluation. In fact, the need to harmonize hazard assessment in the global world has led to the determination of a series of standardized test for several activities, supported by institutions such as OECD and ISO. However, those methods have been traditionally used for chemical substances and are validated for traditional chemicals more than for materials. For some solid materials, a valid approach is to study the substances which are presented in solution, but this approach is insufficient when the particle size and structure is of relevance. NM physicochemical properties can lead to inconsistent toxicological outcomes, even when assessed using well-established in vitro models. These properties—such as high adsorption capacity, pH alterations, distinctive optical characteristics, surface charge, dissolution behaviour, magnetism, and catalytic activity—can interfere with both assay materials and detection systems used in toxicity evaluations.

In vivo assays

In vivo methods expose living animals/plants to the toxicant to assess their effects. They were commonly used in the past for a large variety of toxicological parameters, but their use is declining due to ethical concerns, and they are being substituted, when possible, for different methods, such as those called New-Approach Methods (NAMs). However, they are yet used in different fields.

In vivo tests are the standard approach for ecotoxicity, and in particular for aquatic toxicity. As a general approach, different species are used to cover a range of several trophic levels. Commonly used species for algae are *Chaetoceros gracilis* or *Phaeodactylum tricornutum* as marine, and *Chlorella sp.* or *Raphidocelis subcapitata* as freshwater organisms. For invertebrates, the most common test organism for freshwater systems is *Daphnia magna*, and for marine systems the rotifer *Brachionus plicatilis* and the fairy shrimp *Thamnocephalus platyurus* are commonly used. As for aquatic vertebrates, fishes are the most common test

organisms in *in vivo* ecotoxicity tests, including species such as *Danio rerio, Pimephales promelas, Cyprinus carpio, Poecilia reticulate, Lepomis macrochirus, Gasterosteus aculeatus* or *Oncorhynchus mykiss* (freshwater)*,* and *Cyprinodon variegatus, Dicentrarchus labrax, Pagrus major, Acanthopagrus schlegeli* or *Lutjanus argentimaculatus* (marine). Among those species, some are preferred for standardized tests and, for example, the OECD guidelines 201 and 203 include a list of 5 algae and 11 fish species, respectively, with specific ranges of conditions for the test.

In vivo tests for aquatic toxicity comprise acute exposures with relatively high concentrations over a short period of time, and chronic exposures with generally lower concentrations over a longer period of time. While acute tests are more straight forward in the interpretation of the endpoints (such as survival rate and growth inhibition), chronic tests allow the testing of sublethal concentrations of a toxicant and the corresponding endpoints (reproduction rate), which can be often trans-generational, due to the relatively short life-span of some laboratory test organisms (for example *D. magna*).

For algae, bacteria and other microorganism, which naturally grow under test conditions in a defined medium, growth inhibition assays are applied which focus on the effect of the toxicant in the evolution of the population of a species. The most common example is the algae growth inhibition test, which is commonly used and standardized through guidelines such as the ISO 10253 [1] and OECD 201. For invertebrates, immobilization of the organism after acute exposure to a toxicant is often used as a proxy for mortality, showing the survival of a population after exposure. The most prominent acute test is the *Daphnia* immobilization test guided by OECD 202 [2], but also others such as the marine rotifer toxicity test guided by ISO 19820 [3] are applied. For fishes, the classical fish acute toxicity test is guided by OECD 203 and similar standards. However, this is being substituted by the use of zebrafish embryo, which are promoted as an alternative to the classical fish test and considered a NAM. Those tests have been used both for chemicals and nanomaterials. Finally, chronic effects are studied by using long term *in vivo* tests, such as the *Daphnia magna* reproduction test guided by OECD 211[4], or the Daphnia magna life-cycle toxicity test guided by ASTM E1193.

Despite those tests are considered standard for chemicals, the application of those models to complex materials, particularly in the nano/micro scale presents specific challenges. Several processes affecting the structure of the material occur upon the release of them to the aquatic environment, such as aggregation, dissolution, sedimentation and changes on the ligands [5] Furthermore, the toxic effect of simpler components that can be released cannot be ignored. Therefore, it is recommended to consider the kinetics of the different dissolution processes. This not only affects the actual exposure concentration but can be related to changes in the bioavailability of the MNs in different aquatic organisms [6].

Despite their reduction, several *in vivo* tests involving different kind of mammals as models for human toxicity remain actively enforced and are commonly used. However, in some cases they are only required in certain conditions such as, for example, after an alternative *in vitro* test has been done and if the category (for example the annex in REACH regulation) is higher. An example of this are the tests for skin corrosion/irritation using animals, which are only considered if the production exceeds 10 ton/year and, even then, they are restricted to

cases where the *in vitro* results are not adequate/possible. However, in accordance to the scope of the project, we will focus in the cytotoxicity and genotoxicity.

In those cases, the main advantage of *in vivo* testing is that it considers the systemic toxicity and the results are impacted by the toxicokinetics properties, which relate how the substance is absorbed to the body, distributed, metabolized and excreted. After the systemic exposition, the overall effects of the toxicant can be analyzed (for example in an acute toxicity test, focusing in animal mortality) and histopathological examination of the tissues can be used to observe the damage at the cellular level (similarly to *in vitro* tests). Research on gold NMs unveiled contradictory cytotoxicity results when *in vitro* or *in vivo* tests are used [7]. NMs potential to distribute along different body organs is being studied, and their complex behavior requires further analysis.

Regarding the genetic damage, the European Medicine Agency (EMA) and ECHA recommend both *in vitro* and *in vivo* assays [8],[9]. Based on this recommendation, genotoxicity and mutagenicity may be assessed by the micronucleus test and comet assay, which are suitable to evaluate DNA damage both using *in vivo* and *in vitro* exposition [7]. The comet assay is commonly used in the in vitro approach (described above); however, it also can be performed *in vivo* by cell dissociation from the tissue [10].

In vitro assays

In vitro techniques employ various cell types to evaluate the toxic effects of chemicals or materials upon exposure. The idea is to use cultured cells as a simple model for mechanistic studies to be related to more complex living tissues. Cells can be obtained from real tissue (primary cells) but commonly immortalized cell lines are used for practical reasons. Those cell lines are usually mutated cells which have practical advantages, such as the ability to proliferate and survive in *in vitro* conditions. It is documented that carcinoma cell lines, which are frequently used in laboratory settings for *in vitro* NM toxicity testing, exhibit different pathophysiological characteristics compared to healthy cells. Consequently, the toxicological data derived from such cell lines may not accurately reflect the response of normal cells, leading to potentially conflicting results. Hence, the use of cell lines for toxicity testing requires carefully consideration and validation.

On the other hand, there are techniques used to mitigate the differences between *in vivo* and in vitro tests, such as microfluidic approaches, which enhance the mimmicking of the *in vivo* environment and provide conditions near to those in physiological contexts.

The toxicity assessment of CNM using *in vitro* tests typically involves evaluating cytotoxicity and genotoxicity; which measure significant damage to the cells such as mortality or DNA damage. However, in addition to modelling overall toxicity, *in vitro* studies are also used to explore different adverse effects, as separate issues or as key events in the mechanistic study of toxicology. Some of these studies are not commonly used in regulatory toxicology, but are useful to understand toxicity and in the investigation of its mitigation. In these cases, the effect is often studied by analyzing the changes in the expression of one or more biological molecules involved in the process. Examples of this studies include inflammatory response, and metabolic indicators of toxicity, such as the detection of reactive oxygen species formation, apoptosis, and DNA damage repair.

Must be noticed that those endpoints are not independent. For example, nanoparticles can also induce the generation of reactive oxygen species (ROS), which may alter mitochondrial enzyme activity, subsequently affecting the assay's final readout. Additionally, the absorption spectrum of reduced MTT is pH-dependent, and metal ions can disrupt the MTT reduction reaction. Furthermore, the inherent optical properties of NMs can interfere directly with the readout by increasing light absorption, as demonstrated by sodium titanate nanoparticles [11]. Considering those aspects in the experimental set-up is very important and curation of the data could require check how this was evaluated. For example, the protocols used in CMS for the gathering of primary data (WP3) include cell-free control tests and other mitigation measures to ensure the reliability of the data.

Cytotoxicity refers to the ability of a substance to being toxic to the cells and it is mainly measured by assessing the cell viability (or number of surviving cells). There are several methods existing in the literature for this test, and a few of them are considered standard and offered as commercial kits.

MTT assay is a colorimetric test for assessing cell metabolic activity. It is based on the ability of metabolically active cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to an insoluble purple formazan product. Hence, the amount of formazan produced is proportional to the number of viable cells [12]. This assay has been used to assess the cytotoxicity of nanomaterials like aluminum oxide [13], copper oxide [14], multi-walled carbon nanotubes (MWCNTs) [15], silver [16], zinc oxide and iron oxide in different types of human cells [11].

XTT is another related assay for assessing cell viability and proliferation. In this case, it is based in the cleavage of 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) by dehydrogenase enzymes of metabolically active cells yields a highly orange colored formazan product which is water soluble [17]. This assay has been used to assess the cytotoxicity of nanomaterials like Silver, Cerium dioxide, Titanium dioxide [18], Silica [19] and Gold [20].

Other related assays include, for example, those related with water-soluble tetrazolium salts (WSTs) such as WST-1 and WST-8, which are reduced outside of the cell [21]. Among these, MTT is a positively charged compound that can easily penetrate viable eukaryotic cells, whereas MTS, XTT, and WST-1, being negatively charged, do not enter cells as readily. The interaction of NMs with these assay components can lead to variable results. For instance, carbon nanomaterials have been observed to interfere with these assays by interacting with the components or affecting the readout.

On the other hand, the lactate dehydrogenase (LDH) assay is based in a cytosolic enzyme found in cells. When cells die or are damaged, the plasma membrane becomes permeable and LDH is released into the extracellular medium. Measuring the amount of LDH released into a cell culture can be used as an indirect way to quantify cytotoxicity. This assay has been used to assess the cytotoxicity of nanomaterials like TiO_2 [22], SiO_2 [23], copper and silver [24], MWCNTs [25].

Genotoxicity refers to the ability of a substance to cause damage in the genetic material of the cells. Different methods exist for genotoxicity, according to a recent review, most of the

results for metal oxide NMs correspond to Comet assay (137 of 165 publications analyzed), in comparison with Micronucleus (39), Ames (19) and Chromosome aberration (6) [26].

Comet assay consists of separating intact from fragmented DNA by agarose gel electrophoresis. Broken or relaxed DNA migrates toward the anode faster than undamaged DNA, such that a tail is formed that resembles a comet. The tail length and intensity are indicators of the level of DNA damage caused by the insult. The relative tail intensity (relative to tail plus head) yields a linear correlation with DNA breaks [27]. This assay has been used to assess the genotoxicity of nanomaterials like silver and Al_2O_3 [27], ZnO, TiO_2 [28].

Micronucleus assay is used to detect chromosome damage by identifying micronuclei in interphase cells. It involves exposing cell cultures to a test substance and then observing the formation of micronuclei in cells that have completed nuclear division (micronuclei are formed during the anaphase of the cell cycle from lagging chromosomes or chromosome fragments occurring after chromosome lesions or after chromosome malsegregation) [27]. This assay has been used to assess the genotoxicity of nanomaterials like silver, Al_2O_3 [27], ZnO, TiO_2 [29] and fullerenes [30].

Chromosome aberration is another genotoxicity assay used to detect chromosome and chromatid breaks and other chromosome damage such as translocations as well as alterations in the number of chromosomes [27]. This assay has been used to assess the genotoxicity of nanomaterials like silver [31] and ZnO [32].

The Ames test is a method used for scoring gene mutations using bacteria. It is based on the appearance of colonies formed from amino acid-requiring mutants of *Salmonella typhimurium* or *Escherichia coli* in agar deficient in the amino acid required by the mutant tester strain used. These colonies arise from back mutations of the tester strains, which initially carry mutations in genes required for the synthesis of the respective amino acids [27]. This assay has been used to assess the genotoxicity of nanomaterials like ZnO and TiO_2 [27]. While most tests scoring for NM-induced genotoxicity lead to considerably great numbers of apparently genotoxic NMs, the Ames test yields mostly negative results. It appears advisable to normally refrain from the use of the Ames test for scoring the potential mutagenicity of NMs and to routinely prefer to use mammalian cell mutation assays instead [27].

As mentioned above, in addition of the standardized methods used commonly for regulatory applications, there are other methods based on the assessment of cellular processes related with the adverse effect. An example of these studies is the evaluation of the **DNA damage** through the measurement of the phosphorylated histone γH2AX, which has been detected to follow the DNA double-strand breaks and has potentially a role in its reparation, and thus is used to study genotoxicity. This modification serves as a marker for DNA damage, facilitating the recruitment of DNA repair proteins to the damage site and allowing for visualization and quantification of DNA damage in cells. This assay has been used to assess the genotoxicity of nanomaterials like silver, aluminum oxide, gold and cobalt-chromium [33].

Another example of cellular process related cellular toxicity is apoptosis. **Apoptosis** is a natural and orderly process by which cells eliminate themselves from an organism. Unlike necrosis, apoptosis is not inflammatory and does not damage surrounding tissues. The assays to study apoptosis have been used both for chemicals and NMs, but not always are

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devoted to assessing substance-induced apoptosis but also to evaluate therapeutic or combined effects. From the methods existing in the literature, we remark the following, because apoptosis is a complex process, different biomarkers appear along the process which facilitates the analysis of those on terms of time as early apoptosis (earlier stages in the process) and late apoptosis (later stages).

Annexin V protein can be used as an early apoptosis assay. The detection of apoptosis using annexin V is based on the ability of this protein to bind to phosphatidylserine (PS), a phospholipid that is normally located in the inner leaflet of the plasma membrane in living cells. During the early stages of apoptosis, PS translocates to the outer leaflet of the membrane, becoming exposed on the cell surface. This early exposure of PS on the outer surface of the cell is a characteristic marker of apoptosis and can be detected using annexin V. This assay has been used to assess the apoptosis of nanomaterials like $GdVO_4:Eu^{3+}$, $LaVO_4:Eu^{3+}$ [34], polyurethane [35], CeO_2 [36], silver [37],[38], TiO_2 [39], silica [40], MWCNTs [41], Bi2O3 [42].

Detection of cleaved poly(ADP-ribose) polymerase (PARP-1) is used as a late apoptosis assay. PARP-1 is a 113 kDa nuclear enzyme which is cleaved in two fragments of 89 and 24 kDa during apoptosis. Hence, this cleavage has become a useful hallmark of apoptosis [43]. This assay has been used to assess the apoptosis of nanomaterials like TiO_2 [44], SiO_2 [45],[46], silver [47].

To evaluate cell **inflammation**, different chemokines (such as those of the IL family, TNF-α and MIP) are commonly used as biomarkers of the inflammatory process both to study the induction of inflammation and the anti-inflammatory properties of CNMs. These chemokines have been used to assess the inflammation of NMs like crystalline and amorphous silica, ZnO, titanium dioxide, iron oxide, zinc oxide, carbon nanotubes, fullerenes, and quantum dots [48],[49]. In addition of chemokines, microRNA has been found to be an active biomolecule in the inflammation mechanism, and particularly miR-146a has a key role and it is an excellent marker [50],[51]. Several studies use miR-146a to assess the ability of NMs to counteract inflammation [52]–[54]. However, the results of enzymatic immunoassays can be compromised if cytokines are adsorbed onto NM surfaces, as observed for IL-8 with carbon nanomaterials [55] and IL-6 with metal oxide NMs [56]. This aspect will be considered both in the experimental design and selection of data, for example by measuring the level of the biomarkers avoiding the direct contact with the NM and using replication techniques such as PCR.

Reactive Oxygen Species (ROS) are unstable molecules that contain oxygen and are naturally formed as a product of cellular metabolism. While ROS play an important role in cell signaling and defense against pathogens, their excessive accumulation can lead to oxidative stress, which damages cells and contributes to various diseases. ROS toxicity in cells causes DNA Damage, proteins oxidation, cell membrane damage and apoptosis [57]. 2',7'-Dichlorodihydrofluorescein diacetate (H_2DCFDA) is a compound that penetrates cells and which is a chemically reduced form of fluorescein, used as an indicator of ROS within cells. For example, it can detect the generation of reactive oxygen intermediates in neutrophils and macrophages. Once the acetate groups are cleaved by intracellular esterases and the compound is oxidized, the non-fluorescent H2DCFDA is converted into 2',7'-dichlorofluorescein, which is highly fluorescent. This assay has been used to assess ROS

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concentration caused by NMs like Gold [58], Nickel Ferrite [59], Silica [60], TiO$_2$ [61], Iron Oxide [62], Silver [63], Cerium Oxide [64], GdVO4:Eu3+, LaVO4:Eu3+ [34].

In chemico

A third family of experimental assays are those called *in chemico* which study chemical reactivity of compounds without the need of any cell. For example, the reactivity of a chemical with a particular protein can be measured extracellularly or physicochemical properties can be used to estimate biological effects. However, this are often not differentiated of *in vitro* studies as, for example the Ocular Irritection® test for eye, which is labeled as an *in vitro* test in the OECD guideline 496 and several sources. Those studies are less common and none of them have been found relevant for the endpoints under main consideration and thus, they are included for completion but specific discussion on those methods is not provided.

In silico modelling

In silico approaches are commonly used by chemoinformaticians in several fields to predict relevant properties of chemical substances. For example, they are broadly used in the drug-discovery and toxicology fields to predict the toxicity and biological activity of chemicals. In general, computational predictions are faster and less costly than experimental tests (particularly once a model has been developed and validated). The cost reduction is even more significant compared with *in vivo* tests, with the additional benefit of decreasing the number of animals required and, thus, contributing to the general effort to reduce, refine and replace them for ethical reasons (known as the 3 Rs). Furthermore, computational approaches present a benefit in the development and design phases, as they allow the prediction of properties for unsinthetized or even hypothetical substances.

In this field, QSAR is one of the most commonly used methodologies, due to its advantages such as easy application, potential for mechanistic interpretation and statistical validation. Those models are based in finding quantitative relationships between the structure of a chemical and its properties. Thus, a key component is to describe the structure as a series of numerical descriptors that represent it. QSAR models for organic chemicals are a mature technology widely accepted for regulatory bodies and with hundreds of commonly used public and commercial models. However, the evolution to the QSAR methodology to be applied to materials and, in particular, to NMs is a newer advance and an active field of research. Their advance is hindered due to the intrinsic difficulty of the structural characterization of the structure of the material which leads to the lack of quality data available to their generation.

There are several adaptations to QSAR models in order to apply them to nanomaterials, which we will as nanoQSAR in this deliverable; even if they have received other names such as Quantitative Nanostructure-Activity Relationship (QNAR), Nano-QSAR, NanoSAR, Nano-QFAR, etc. The first described nanoQSAR model is from 2009 [65], but the number of nanoQSAR relevant models is growing significantly [66]–[70]. One reason of their expansion is the exploration and design of new descriptors adapted to the materials structure and the inorganic nature of most NMs. Another reason is the progressive generation of much more experimental information on NMs and the efforts in the harmonization of the characterization and assessment methods, with aims to their application at regulatory level.

A review of *in silico* preexisting models in the toxicity of NMs was done and a series of models is summarized in the *Table 1*. The data in the table mainly refers to nanoQSAR models (including advanced multi-target models and perturbation approaches), but other in silico approaches such as Bayesian networks are also included.

Table 1. List of papers with relevant computational models for nanomaterials

Endpoint	Target	MOx	Metal	Carbon based	Mixture /other
Apoptosis	Multiple				[71]
Cell Viability	Human	[72]–[79]	[79]	[74],[80],[81]	[74],[75],[82]–[85]
	Porcine	-	-	-	[85]
	Murine	[74],[76],	-	-	-
	Multiple	[86]	-	-	[87]
Cell Uptake	Human	[71],[88]–[90]	-	-	[80]
Cytotoxicity	Bacteria	[65],[80],[91]–[109]	-	-	[80]
	Human	[97],[100],[101],[103],[105]–[116]	[117]	-	[118],[119]
	Murine	[101],[111],[112],[114],[117],[120],[121]	[117]	-	[119]
	Virus	-	-	[122],[123]	-
	Daphnia magna	[124]	-	-	-
	Saccharomyces cerevisiae	-	[125]	-	-
	Fish	-	[126]	-	-
Ecotoxicity	Multiple	[127]	[127]		
EZ metric	Danio rerio	-	-	-	[128]
Genotoxicity	Multiple	[129],[130]	-	-	-
Immobilization	Daphnia magna	[131]	[131]	-	[131],[132]
Inflammatory potential	Human	[133]	-	-	-
Interaction energy	SARS-Cov-2	-	-	[134]	-
Luciferase	Murine	[135]	[135]	-	-
Membrane damage	Human	[136]	-	-	-
Mutagenicity	Bacteria	-	-	[137]–[140]	-
Oxidative stress	Human	[141]	-	-	-
Photodegradation	Murine	[120]	-	-	-
	Multiple	[102]	-	-	-
Toxicity	Human	-	-	-	[88]
	Multiple	[127],[142]	[127],[142]	-	-
	Danio rerio	[100]	-	-	-
	Aliivibrio fischeri	[143]	-	-	-
Hatching Enzyme ZHE1	Danio rerio	[69]	-	-	-

Zeta Potential	Human	[144]	-	-	-
	Multiple	[102]	-	-	-

Regarding the predicted properties, most published models are devoted to toxicity in humans, modeled using in vitro approaches with targets such as bacteria and mammal cells. In this case, the most common endpoint is cytotoxicity, but also many genotoxicity studies are found. Regarding environmental endpoints 3 publications in *Daphnia magna*, 1 in zebrafish and 4 with different targets). Related to the parameters studied in WP3, we have also identified predictive models focused on the inflammatory and oxidative potentials. On the other hand, a few examples of nanoQSAR models relating other properties are found, such as bioactivity against virus (protein binding) and physicochemical properties [66].

Regarding the composition, most of the models are based on metal oxides (MOx), both solely or as a part of a wider dataset. Noble metals are also commonly found, but in those cases it is common to find that the focus is on the coating and not in the compositions. Other core components found in publications are SiO_2, Cd-based quantum dots (QDs) and carbon-based inorganic materials such as fullerenes, carbon nanotubes and graphene flakes [66].

We did not find any model regarding biodegradability for nanomaterials, despite identifying a few publications regarding modelling that include relevant keywords. However, biodegradability is not among the predicted properties but, in some cases a general comment about the properties of the materials included in that particular study. This is not unexpected because the models in materials are usually focused in particular families and thus usually only inorganic materials are considered. However, biodegradability cannot be directly applied to those materials as biological degradation is not expected, even if the materials can be removed of the media (for example by aggregation, dissolution or passivation of the surface). The biodegradability of inorganic carbon-based NMs such as fullerenes and carbon nanotubes and organic polymers such as cellulose has been investigated experimentally, confirming that only the organic ones were readily biodegradable [145].

Finally, in addition to the analysis of the publications, that are the vast majority in the field, we have search for QSAR models available as computational tools (online servers and tools). There is a significant amount of commercial and free services that use QSAR models to predict different properties for molecular substances. Vega, Danish (Q)SAR

Table 2. Summary of available QSAR software for molecular chemicals

Software	Predicted properties	Link
ProtoPRED	Phys-chem, environmental fate and distribution, toxicokinetics, ecotoxicity and human toxicity	https://protopred.protoqsar.com/
Vega	Phys-chem, environmental fate and distribution, toxicokinetics, ecotoxicity and human toxicity	https://www.vegahub.eu/portfolio-item/vega-qsar/
TEST	Phys-chem, ecotoxicity and human toxicity	https://www.epa.gov/comptox-tools/toxicity-estimation-software-tool-test
KATE	Ecotoxicity	https://kate.nies.go.jp/
MLTOX	Phototoxicity & genotoxicity	https://mltox.fiit.stuba.sk/

EPISUITE	Phys-chem, environmental fate and distribution	https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface#what
ADMETlab	Phys-chem, toxicokinetics and human toxicity	https://admetlab3.scbdd.com/server/screening
STopTox	Human toxicity	https://stoptox.mml.unc.edu/
DanishQSAR	Environmental fate and distribution, toxicokinetics, ecotoxicity and human toxicity	https://qsarmodels.food.dtu.dk/
ECOSAR	Ecotoxicity	https://www.epa.gov/tsca-screening-tools/ecological-structure-activity-relationships-ecosar-predictive-model

However, in the case of nanomaterials, there are very few tools available. We have compiled a list of predictive models for (nano)materials which are available online in different platforms:

- ProtoNANO (https://protopred.protoqsar.com/ProtoNANO_info)

ProtoNANO is the NM-focused module of ProtoPRED (a prediction platform owned by PQSAR). This module offers some nanoQSAR models developed as part of a MSCA-IF European Project (NanoQSAR) related with different endpoints such as cytotoxicity (in bacteria, human cells and tumoral cells), zeta potential and partition coefficient.

- Online chemical database (OCHEM): https://ochem.eu/

This database includes a modelling environment and allows users to share their models. Despite data in OCHEM is basically based in organic molecular substances, 15 different models have been found identified as NM-based, all of them related with aquatic toxicity. However, some are alternative approaches to the same property, and thus share a database, so the number of real different endpoints and reusable data is less.

- NanoSolveIT (https://nanosolveit.eu/resources/tools-services/)

As part of this H2020 project devoted to the toxicity of nanomaterials a series of tools were developed, including tools related to study the exposure and uptake of nanomaterials. Regarding the parameters of interest, there are two nanoQSAR models for the cytotoxicity of metal oxides: a regression model that predicts cell viability values and a qualitative model. The quantitative model focuses on ATP or LDH assays (being the assay a descriptor that affects the prediction). In both cases, the models are trained with data on BEAS-2B and RAW 264.7 cells (plus E. coli for the qualitative). In all cases the model requires a significant amount of data in addition to basic characterization such as size, such as the chemical potential, enthalpy of formation and energy of the conduction band. Although there is a tool related to the toxicity to *Daphnia magna*, this is not related with the scope of this review, as it is an image detection.

- Enalos Cloud (https://www.enaloscloud.novamechanics.com/all.html)

Enalos Cloud is a server with different computational tools, including a few devoted to nanomaterials such as a constructor of unique identifiers for nanomaterials (NInChi), tools to prepare geometries for atomistic models and models that obtain descriptors from images. Regarding toxicity prediction, it provides several models including the models discussed above for NanoSolveIT. As examples, there are read-across approaches for ecotoxicity of

silver, TiO$_2$, and Ag$_2$S nanoparticles identified by their charge, tested media, TEM and DLS sizes and the concentration. Finally, it has a model for iron-based nanoparticles based in experimental descriptors such as the relaxivities and zeta potential.

- NanoDesk (currently unavailable)

NanoDesk, a finished Interreg-Sudoe European project, produced a platform to grant access to their outcomes, including a QSAR online tool and access to the datasets. The platform included different models for genotoxicity, cytotoxicity (LC50), cytotoxicity (LOEL), bioaccumulation in Daphnia magna and a cytotoxicity model combining 5 endpoints (CC50, EC50, IC50, LC50, TC50). Unfortunately, the webservice is no longer accessible but we had access to the data. From this project, a dataset was compiled with 3320 cell viability values, 171 genotoxicity values, 68 ecotoxicity values and 658 physicochemical properties values, mainly for metal oxides.

Selection of parameters and data availability

Summarized results of the data mining process

Data-based models, as those proposed in WP5 are built from the information obtained from previous, validated experimental data. Thus, compiling and curating data is an essential task that will expand along the model development process. In this section, we summarize the findings of the first phase of the overall data-mining process, focusing in the selection and compilation of data to be used to develop models in the WP5.

The objective is to explore the vast amount of potential toxicity data, including several sources and potential parameters, to finally define a few curated datasets for particular models. Hence, it can be described as a 3-step process where successively we filter the information to respond a particular question (Figure 1). In first place, the parameters of interest are selected based on the potential interest for modelling and the adequacy to the project objectives. In second place, data is filtered according to the experimental conditions reported and the adequate tests. Finally, a subset of consistent data (same value and units) is selected and curated for model development.

Figure 1. Graphical representation of the steps for selecting the parameters to model

Parameters
- A property or biological activity
- Cytotoxicity
- Selected by regulation and project objectives

Tests
- Experimental conditions
- MTT assay in EA.hy927 cells
- Selected in basis to data availability

Endpoints
- Particular value
- EC$_{50}$ (different cells)
- Compromise data consistency and quality
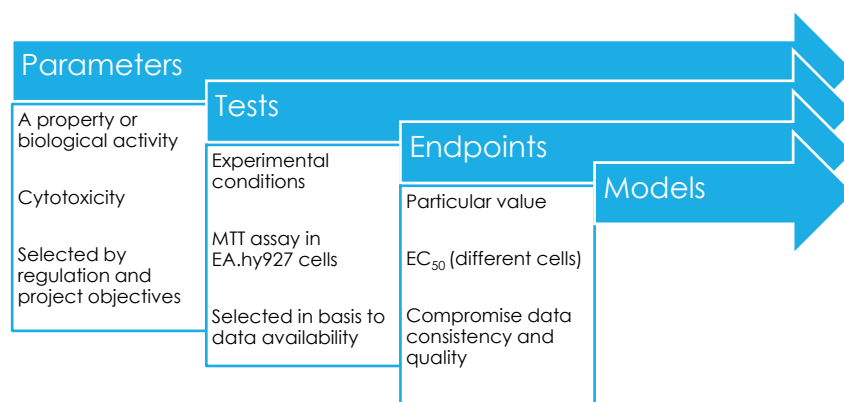
Models

In this section we will describe the sources and methods used for data-mining and discuss the progress up to this moment for each of the selected parameters. Even if additional data can be gathered for modelling purposes, the discussion about data availability and variability at this point will be useful to guide our efforts and focus on more promising models.

Data sources / Methodology

The data mining process has consisted in compiling information on different data sources, including curated databases and scientific publications. Databases search include both general databases with a clear majority of chemical data and other that are focused in nanomaterials. Because the CMS project includes the collection of a database as part of WP7, database analysis did not always include the compilation of data, but just its analysis in basis to summary information and or preliminary queries. The actual compilation and management of the data is planned to be performed in collaboration with WP7.

Collection of relevant publications have combined the exploration of documents provided by the partners project involved in the selection of the materials in WP2 and the search of new publications in scientific searchers such as Google Scholar, Scopus, PubMed and ScienceDirect. A wide range of keywords have been used including information about the kind of materials (such as nanomaterials, nanoparticles, nanoform, TiO2, cellulose, silver, …) including full words, common abbreviations and chemical formulas. On the other hand, the different parameters have been searched both by using broad terms (toxicity, genotoxicity, apoptosis), as well as including the assay name (Comet, MTT, micronucleus) or key chemicals involved in the test (PARP). Additional keywords have been used to deeply explore certain endpoints such as particular cell types and organism species. After revision of several publications, a list of papers has been selected as potentially including relevant data or key information for modelling the selected parameters (further details below).

In addition to the keywords necessary to identify that the assay corresponds to parameter of interest. For example, in some cases additional filters have been required to identify the role of the substances as toxicity inducers or inhibitors/protectors. That difference has a potential relevant for our analysis because the characteristics of the experimental studies are very similar, but the ability of the inhibition data to train models to predict toxicity is not clear. On one hand, all the studies are relevant to discuss the availability of the techniques, as they are commonly used to assess the adverse effect. On the other hand, treatment-targeted studies which often (a) combine different substances in the same test and (b) use very active toxicants in large doses as toxicity inducers. Meanwhile this data could be used for modelling inflammatory potential in the presence of an inducer, it is not applicable to model the direct inflammation caused by CNMs.

Overall data availability

In the first place, a global search of available scientific literature on the parameters of interest has been performed. Several searches were done to explore the field, but as a summary of the process, the number of publications found in a series of searches in PubMed are presented in

Table 3. In this first approach, we have not explored the kind of chemical or material involved, but how the parameter was measured using a general keyword for the parameter plus additional keywords to specify the assay type. In addition of the general results, we have

refined the search by adding cell lines such as EA.hy926, U-937, HUVEC, HepG2 and A549. This list includes the cells selected for performing the experiments in CMS, in basis to their quality, reliability and adequacy for the adverse effects studied. It also includes data related to primary HUVEC cells, as they are endothelial cells to which the EA.hy926 cells are very similar. Due to its stability and other properties, the hybrid EA.hy926 is the best option for testing, but the availability of preexistent data is limited. For comparison, we have also included a couple of tumor-based cells which have been commonly used for bioassays, but that are not adequate for the toxicity and mechanistic studies proposed in CMS.

Table 3. Number of publication in PubMed

Parameter	Test	Any	EA. hy926[a]	U-937[b]	HUVEC	HepG2	A549
Cytotoxicity	--	403182	147	1948	1950	10155	11797
	MTT	24216	24	167	307	1863	1919
	XTT	820	-	14	5	34	31
Genotoxicity	--	41263	6	6	52	887	557
	Comet	6681	3	1	21	420	211
	Micro-nucleus	6696	1	-	8	255	92
Apoptosis	Annexin	22689	22	275	331	734	958
	PARP	13278	10	202	113	451	617
	--	602203	214	3519	4514	9557	1231
Inflammation[d]	--	1635507	304	2549	5144	3370	4439
	TLR4	20473	6	99	152	81	106
	IL6	131129	65	412	*938*	593	896
	MiR-146	157	1	1	*4*	-	1
	IRAK1	838	-	6	*14*	6	8
ROS[e]	--	414660	253	868	2860	4443	3656
	H_2DCFDA	2819	10	17	*77*	55	70

A Search by EA.hy926 or hy926

B Search by U-937 or U937

C Search by apoptosis or apoptotic

D Search by (inflammation or inflammatory)

E Search by ROS or "oxidative stress"

Similar searches were done also in Google Scholar and Scopus, which provided analogous searches. Both sources provided more publications than PubMed, for example the search for cytotoxicity using MTT in any cell provides 24216 publications in PubMed but 85543 in Scopus and 376000 in Google Scholar. Similarly, if PubMed did not identify any publication with XTT and the EA.hy926 cell, 7 publications are found in Scopus and 333 in Google Scholar. As seen in this example, the relative proportion of data among different searches is similar to that show in the table above, so such detail is not presented here. Must be noticed, however, that the larger amount of information provided (particularly in Google Scholar) implies the need to apply more strict filters to sieve useful data (for example with specific searches for material types or additional testing details) and, nevertheless, several papers including the keywords in a non-relevant way are found and it requires the human inspection of the papers. Nevertheless, because these sources provided more data, they have been used for searching most of the publications discussed along this text, using a larger variety of keywords and filters than those explicitly discussed in this deliverable.

The results of this research show the number of publications related to the field, but do not ensure that those publications can provide useful data for modelling, as it would require case-by-case analysis of the publications. They are several reasons for a publication do not provide useful data including the following kind of publications:

- Papers with a qualitative discussion of the mechanism but not specific data
- Papers that explored adverse effects in very specific conditions, including combination of different substances, cells, infections and other effects in the same study
- Papers devoted to the study of treatments and therapeutical approaches which do not provide relevant untreated, toxicity data.
- Papers with results of a different parameter/test/cell but that include discussion regarding the searched one as alternative
- Reviews (which could provide curated data, but duplicate with primary sources)
- Papers that have been retracted or use deprecated methodology

Hence, data from papers is challenging to compile and analize, and it is more prone to lead to inconsistent databases and errors. Thus, we also performed searches in databases which allow the retrieval of curated data and, in some cases, include specific fields for the cell-line and other parameters, such as our first example: the ChemBL database. The data presented in Table 3 relates with the search in ChemBL by bioassays and the number of compounds is the aggregated raw data of the search (note that this is not curated and it could include duplicate or inadequate values).

For each assay, an initial search has been done in basis to keywords related to the assay (for example, "comet" for the genotoxicity comet assay and "H2DCPFA" for the ROS test) and the aggregated number of compounds is labeled as "all compounds". Then, a second manual step was applied to remove those tests related to inhibition and/or protection assays, by reading to the description (as well as other assay descriptions which seem to be not adequate) to select only the bioassays that study the hazard induction. In Table 3, it can be noticed that there is a significant reduction of the data, particularly for the inflammation tests, which are mostly for anti-inflammatory studies. Despite the same method can be used to both aims, as it is a valid study of the inflammation, data regarding treatments is not useful for modelling, as the inducer of the damage is often selected among a handful of options and it is applied in high doses. Filtering by cell lines reduces significantly the results and for several of the cell-line/test combination no results are found in this database.

Table 4: Preliminary results of data mining in ChemBL

Parameter	Test (keyword)	All compounds	Compounds hazard	Bioassays hazard
Cytotoxicity	MTT	554806	337225	38200
	XTT	20012	8840	1034
Genotoxicity	Comet	856	769	414
	Micronucleus	532	350	249
ROS	H2DCPFA	1094	843	297
Apoptosis	Annexin	113	103	66
	PARP	2541	2433	1601
DNA damage	H2AX	756	478	272
Inflammatory	TLR4	2199	114	32
	IL6	9308	651	156
	mR-146	1463	342	58

	IRAK1	2118	1550	29

In summary, it can be observed that the availability of data is very variable depending on the search. However, in all cases, even if there is a significant amount of data on those properties, it is not commonly found with the cell cultures proposed. For example, even if the general availability of cytotoxicity data looks huge, when the test and cell-line is restricted it decreases significantly.

The same trends were found in other data sources. A similar search in PubChem was performed but for most of the cases only data from ChemBL was found and not significant differences were appreciated in the relative amount of data. On the other hand, a search in the online chemical database ochem.eu, only finds 8 values related to U937 cells, and those are values for IC_{50} against cell proliferation. In that case, no values were found for the EA.hy926 cells. Otherwise, removing the cell filter, we obtained 441 cytoxicity records (related to cell lines such as SHSY5Y, SK-N-MC and NB1).

Regarding the ecotoxicological endpoints, we also conducted a search (*Table 5*) for various organisms in the ChemBL database, the REACH database from ECHA (retrieved through QSAR Toolbox, https://qsartoolbox.org/) and in ECOTOX from the EPA (https://cfpub.epa.gov/ecotox/explore.cfm). This search shows that most of the available data is related with *D. magna* and that, for those endpoints, regulatory-based databases such as those from ECHA and EPA have more data.

Table 5. Data availability of toxicological data regarding different aquatic species

Species	ChemBL	REACH	ECOTOX
Tetrahymena thermophila	75	28	1263
Brachionus calyciflorus	49[a]	5315	1912
Thamnocephalus platyurus	4	76	326
Selenostrum capricornutum	39	356	1122
Daphnia magna	75	59079	35636

[a] The search was done by the phylum (rotiphera), as data was not recorded by the species.

However, most of this data is based on organic molecular compounds, and WP5 modelling involves also a series of nano and micromaterials (both organic and inorganic). Thus, additional exploration of nanomaterial-based databases was done and a summary of the results are presented in *Table 6*. Data from different EU-funded projects was gathered using the eNanoMapper platform. NanoE-Tox data, collected by the National Institute of Chemical Physics and Biophysics, was obtained as an spreadsheet supporting its publication.[146]

Table 6. Data availability for different materials classes in the databases

Source	Cytotoxicity	Genotoxicity	ROS	Inv.	Algae
eNanoMapper	TiO_2 (298), Ag (50)		TiO_2 (334), Ag (62)	TiO_2 (210),	
NANoREG*	*TiO_2 (2250), Ag (1788), Cell. (161), Chem (84)*		TiO_2 (1943), Ag (1243), Cell. (183), Chem (113)	TiO_2 (72), Ag (8)	
anoReg2*	*TiO_2 (266), Ag/Au (98)*		TiO_2 (4), Ag/Au (19)	TiO_2 (1), Ag/Au (363)	
caLIBRAte*	*TiO_2 (37), Ag (18),*		*TiO_2 (6), Ag (19),*	*Chem(7)*	

	Chem(761)		*Chem(20)*		
GRACIOUS*	Chem (774)			Chem(593)	
NanoE-tox				TiO₂ (118), Ag (149), NM (167)	TiO₂ (41), Ag (8), NM (120)

Abbreviations: Cell.: Nanocellulose, Chem: Chemicals, NM: Other NMs
Italic values in the cytotoxicity column correspond to Cell viability.
* Accessed through eNanoMapper

Analysis of the data availability by parameter

In this section, we briefly present a summary of the data found for the parameters to be studied and present a table with the most relevant data sources, classified according to different aspects such as the kind of CNM, the assay conditions or the specific kind of data available (endpoint).

Table 7. Summary and classification of data sources regarding cytotoxicity

Criteria	Class	Papers	Databases
Material	Metallic	[21],[147]–[160]	eNanoMapper, NANoREG, NanoREg2, CaLIBRAte
	Polymers	[149],[161],[162]	
	MOx	[160]	eNanoMapper, NANoREG, NanoREg2, CaLIBRAte
	Cellulose	[163]	
	Chemicals	[164]	ChemBL, NANoREG, CaLIBRAte, GRACIOUS
Test	MTT	[21],[148],[157],[158],[164],[165]	eNanoMapper
	XTT	[150],[166]	
	Other/not-identified	[155],[159]	NANoREG, NanoREg2, CaLIBRAte
Endpoint	Cell viability	[158],[166],[167]	NANoREG, NanoREg2, CaLIBRAte
	EC50	[21],[148],[150] [149],[161],[162]	
Cell line	VK2-E6/E7	[155],[158]	
	GMK-AH1	[159]	
	EA.hy926	[150],[168],[169]	
	U-937	[166]	
	Hep2G	[149],[161],[162]	
	HUVEC	[167]	

Table 8. Summary and classification of data sources regarding genotoxicity

Criteria	Class	Papers	Databases
Material	Metallic	[21],[170]	eNanoMapper, NANoREG, NanoREg2, CaLIBRAte
	Polymers	-	-
	MOx	[21],[147],[171]–[173]	eNanoMapper, NANoREG, NanoREg2, CaLIBRAte
	Cellulose	-	-
	Chemicals	[174]–[179]	ChemBL, NANoREG, CaLIBRAte
Test	Comet	[21],[148],[150],[180]–[182]	
	Micronucleus	[183],[184]	
	Ames	[175],[183]–[185]	
Endpoint	Binary classification	[182],[186],[187]	
Cell	Caco2	[21]	
	HepG2	[21]	
	BEAS-2B	[147],[188]	
	SH-SY5Y	[171],	
	U-937	[150],[180]	

Table 9. Summary and classification of data sources regarding oxidative stress

Criteria	Class	Papers	Databases
Material	Metallic	[58],[60],[63]	NanoREG, NanoREG2, GRACIOUS
	Polymers	[61],[189]	
	MOx	[34],[59],[61],[62],[64]	NanoREG, NanoREG2, GRACIOUS
	Cellulose	[189]–[191]	
	Chemicals	[61]	NanoREG, NanoREG2, GRACIOUS, ChemBL
Test	H_2DCFDA	[34],[58]–[64],[190],[191]	NanoREG
	Oxiblot® kit, Merck		NanoREG2
	NRF2ACTIVATION		GRACIOUS
Endpoint	PERCENTAGE_OF_CONTROL		NanoREG, NanoREG2
	IC50		NanoREG
	CARBONYLATION		NanoREG2
	LUCIFERASE_ACTIVITY		GRACIOUS
Cell	A549	[58],[59]	NanoREG, NanoREG2
	THP1		NanoREG
	CACO-2		NanoREG
	3T3		NanoREG
	HepG2		NanoREG, ChemBL
	NRK-52e		NanoREG2
	HEK293		GRACIOUS
	HUVEC	[60]	

Table 10. Summary and classification of data sources regarding inflammatory response

Criteria	Class	Papers	Databases
Material	Metallic	[166],[192]	NanoReg
	Polymers	[193],[194]	NanoReg
	MOx	[166],[195]	NanoReg
	Cellulose	[196]	NanoReg
	Chemicals	[168],[169]	NanoReg
Marker	IL-1	[166],[192],[194],[197]	NanoReg
	IL-5	[196]	
	IL-6	[155],[159],[168],[169],[194],[195],[198]	NanoReg
	IL-12	[196]	NanoReg
	IL-10		NanoReg
	IL-8	[169],[193]	
	TNFa	[159],[166],[168],[192],[194],[197],[198]	NanoReg
	PGE2	[192]	
	MIP1	[196]	
Endpoint	Marker concentration	[168],[192],[194],[197]–[199]	NanoReg
Cell	pBMEC	[192]	
	Microglial cells	[195]	
	HaCat	[155]	
	VK2-E6/E7	[155]	
	A549		NanoReg
	THP-1		NanoReg
	RAW 264.7		NanoReg
	EA.hy926	[168],[169],[193],[197],[199]	
	U-937	[166],[194],[198]	

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Table 11. Summary and classification of data sources regarding apoptosis

Criteria	Class	Papers	Databases
Material	Metallic	[200]–[204]	
	Polymers	[205]	
	MOx	[206],[207]	
	Cellulose		
	Chemicals	[202],[206],[208]–[211]	ChemBL
Biomarker	Annexin-V	[200]–[202],[209],[210]	ChemBL
	PARP		ChemBL
Cell	EA.hy926	[200]–[202],[205],[209]	
	U937	[203],[203],[206]–[208],[210],[211]	

Table 12. Summary and classification of data sources regarding aquatic invertebrates

Criteria	Class	Papers	Databases
Material	Metallic	[212]–[216]	NanoReg , NanoE-Tox
	Polymers	[217],[218]	
	MOx	[219]–[223]	NanoReg, NanoReg2, NanoE-Tox
	Cellulose		
	Chemicals	[215],[216],[224]–[229]	NanoReg, NanoReg2, ChemBL
Species	Brachionus calyciflorus	[213]–[215],[218],[222],[223],[225],[226]	ChemBL NanoE-Tox
	Thamnocephalus platyurus	[216],[227]–[230]	ChemBL NanoE-Tox
	Daphnia magna	[212],[214],[217]–[221],[226]	NanoReg, NanoReg2, ChemBL, NanoE-Tox
Endpoint	EC50	[212],[219],[221]	NanoReg, NanoReg2
	EC10	[213],[219],[220]	NanoReg2
	LC50	[212]–[214],[216],[217],[221],[222],[225]–[230]	NanoReg
	Long-Term	[212],[219],[220],[224]	

Table 13. Summary and classification of data sources regarding growth inhibition of algae and related species.

Criteria	Class	Papers	Databases
Material	Metallic	[231]–[237]	NanoReg, NanoE-Tox
	Polymers	[232],[238]	
	MOx	[231],[232],[238]–[241]	NanoReg, NanoReg2, NanoE-Tox
	Cellulose		
	Chemicals	[232],[234],[236],[239],[240],[242]–[249]	NanoReg, NanoReg2, ChemBL
Species	Tetrahymena thermophila	[232]–[234],[237]–[239],[242]–[244]	ChemBL NanoE-Tox
	Selenastrum capricornutum (Raphidocelis subcapitata/ Pseudokirchneriella subcapitata)	[235],[236],[240],[241],[245]–[249]	NanoReg, NanoReg2, ChemBL NanoE-Tox
Endpoint	EC50	[231],[242]	NanoReg, NanoReg2
	EC10		NanoReg, NanoReg2
	LC10		NanoReg
	LC50	[239]	NanoReg

	IC50	[233]–[238],[240],[241],[243]–[249]	

Conclusions and modeling plan
Main conclusions of the data review and consequences for the research plan

In this deliverable we have reviewed the literature regarding experimental and computational assessment of toxicity and biodegradability of chemicals and, particularly, materials in the nanoscale. After the revision of the data discussed above, we reach a series of conclusions that can be summarized in the following principal points:

- Data availability varies significantly among parameters

There is a big difference in the availability of data for the main toxicological endpoints, such as cytotoxicity, genotoxicity and ecotoxicity, and other endpoints such as apoptosis and inflammation. A potential cause, is that meanwhile some toxicological parameters are commonly requested for regulatory purposes by ECHA, EFSA, EMA and other institutions, other studies are mainly devoted to the scientific assessment of mechanistical toxicology and therapeutic applications. The existence of the later approach (evaluation of therapeutic effects) has a huge effect in data quantity and quality. Because the methods to study hazard and potential hazard mitigation are the same, there is a larger number of data sources available, but not all the information is relevant to create a database regarding toxic effects.

- Data for NMs is more difficult to obtain, and requires a more thoughtful curation

The data-mining efforts confirm the hypothesis that NMs data is significantly scarcer. There are less databases related to nanomaterials and, in general, there is no data on those materials in classical databases. Even sources with potential nanoscale data cannot be obtained from those sources, as the characterization of the materials is not properly documented. For example, ECHA database includes particle size information, but nanoforms are not labeled separately. Hence, it is not straightforward to stablish a reliable link between which activity data correspond to each particle size.

On the other hand, there is no a systematic approach for characterization. For example, almost all sources in nano- and microparticles includes the size; but several different assays can be used, not always comparable as there are significant differences between them.

- *In vitro* data is very variable in terms of cell-lines and assay conditions.

For all the parameters under discussion, we have searched the availability of data for several conditions, including the general overview and specific conditions. In general, we have observed a significant variance among the cell lines used for testing, including those selected in the WP3 and other options. The revision of the state of the art suggest that the cell line could have a significant effect, but the lack of consistency makes impossible to gather a single-cell database. On the other hand, the differences among assays are more significant and the most common approach is to prepare single-test models. Thus, these two factors should be of relevance in the development of models and they will require a deep analysis of the preexistent and generated data. This task, in fact, expands to WP3, whose experts will

also have in consideration this analysis to guide and assess the selected techniques, as well as provide guidance in the pros and cons of the different cell-lines and assays and the main characteristics required to assess the reliability and usability of data for modeling. In this sense the CMS action will serve to collate a primary database of very consistent toxicological data for a diverse family of chemicals and materials, which is a key requirement for better modeling.

In conclusion, the modeling plan for WP5 has been designed in the following way. It must be noticed that this is a plan that can be revisited during the progress of WP5 depending on further data acquisition, preliminary modelling and new scientific findings inside or outside the consortium. Also, the plan is not a restricted list of models to be performed but a prioritization of interesting endpoints.

A first impact of the data-mining process in the modelling plan is that it has confirmed that selecting the appropriate data is not a simple task. On one part, there is no single, massive databases that can be used, particularly for materials, and thus it would require analysis of publications and collection of data from different sources. The step of selecting and curating the data is essential in all the modelling tasks and it would be necessary the cooperation of different partners to understand the complexity and variety of data and to select the adequate data. Additionally, statistical techniques and preliminary modelling steps will be performed to assess the potential of different curation criteria.

Regarding task 5.3, the *in silico* models for nanomaterials and chemicals, the priority will be given to models for the direct toxicity parameters, which seem to have more consistent and available data. Those parameters are:

- Cytotoxicity
- Genotoxicity
- Algae growth inhibition
- Invertebrate acute toxicity

In all cases, the data will be selected to be compatible with the results provided by WP3, but not limited to those in order to have better databases. In this sense, the effect of including external data related with alternative target cells/species in the modelling database, will be also evaluated. This would increase the data availability and potentially provide a more general model but has the risk of introducing inconsistency, reducing the quality of the model.

Data from the other *in vitro* tests explored in this deliverable would be also compiled, but due to the difficulties in finding data they will be mostly explored as potential input for the model (i.e. as simpler experimental parameters that can be used to estimate toxicity) than as a parameter to be predicted. Finally, the consistent data obtained from WP3 and the insight of the experts in the field will be used to analyze the more diverse data and discuss its applicability.

On the other hand, because the availability of both preexisting models and data is higher for chemicals, we will try to obtain models for all the parameters discussed here. Thus T5.2 will include, at least, the preliminary steps to assess the viability of creating new models, i.e. to compile a specific database, curate it and analyze the data; to calculate molecular descriptors and to check the statistical relevance of those. This process will create potential

models to be used to assess chemicals by themselves or as a part of combined material-based models of T5.3. Furthermore, the findings from molecular-based models could be used to discuss aspects such as the influence of the cell type and assay conditions in the models, contributing to guide the selection and curation of data for the subsequent models on materials.

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