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## Nanotoxicology data for in silico tools: a literature review

Item Type	Article
Authors	Furxhi, Irini;Murphy, Finbarr;Mullins, Martin;Arvanitis, Athanasios;Poland, Craig A.
Citation	Nanotoxicology; 14 (5), pp. 612-637
Publisher	Taylor & Francis - Routledge
Download date	2026-04-17 21:46:54
Item License	<a href="https://creativecommons.org/licenses/by-nc-sa/1.0/">https://creativecommons.org/licenses/by-nc-sa/1.0/</a>
Link to Item	<a href="https://hdl.handle.net/10344/8567">https://hdl.handle.net/10344/8567</a>

1 **Nanotoxicology data for in silico tools. A literature review.**

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## 19 **1 Abstract**

20 The exercise of non-testing approaches in Nanoparticles (NPs) hazard assessment is necessary  
21 for the risk assessment, considering cost and time efficiency, to identify, assess and classify  
22 potential risks. One strategy for investigating the toxicological properties of a variety of NPs is  
23 by means of computational tools that decode how nano-specific features relate to toxicity and  
24 enable its prediction. This literature review records systematically the data used in published  
25 studies that predict nano (eco)-toxicological endpoints using machine learning models. Instead  
26 of seeking mechanistic interpretations this review maps the pathways followed, involving  
27 biological features in relation to NPs exposure, their physico-chemical characteristics and the  
28 most commonly predicted outcomes. The results, derived from published research of the last  
29 decade, are summarized visually, providing prior-based data mining paradigms to be readily  
30 used by the nanotoxicology community in computational studies.

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## 34 **Keywords**

35 *Nanoparticle; nanotoxicology; machine learning; in silico;*

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## 40 2 Introduction

41 Nanotechnology is one of the main 21<sup>st</sup> century emerging technologies with enormous  
42 potential for innovative applications, estimated to have an economic impact reaching \$90.5  
43 billion by 2021 (McWilliams, 2017). It affects multiple applications and products leading in a  
44 rapid proliferation in the number of commercially exploited nano-embedded materials.  
45 However, NPs display high heterogeneity regarding their physicochemical (p-chem) properties  
46 in relation to toxicological effects, narrowing risk assessment for NP to an ad hoc testing  
47 process. The p-chem properties of NPs form their functionality and influence their  
48 environmental spreading, biological absorption, dissolution and (eco)toxicity (Giusti, *et al.*,  
49 2019). Traditional hazard assessment relies mostly on the use of *in vivo* testing, which poses  
50 technical challenges when extrapolating to humans and ethical dilemmas and requires time and  
51 resources (Chen, *et al.*, 2018). For these reasons, *in silico* methods have gained increasing  
52 popularity in the field of nanotoxicology in accordance with the 3R (Replacement, Reduction  
53 and Refinement) principles of diminishing *in vivo* toxicological studies. However, *in silico* tools  
54 are not yet accepted by regulators as a stand-alone solution but as complementary tools  
55 (Burgdorf, *et al.*, 2019, ECHA, 2017). Implementing such alternative approaches in hazard  
56 assessment is defended by the Registration, Evaluation, Authorization and Restriction of  
57 Chemicals (REACH) regulation as exploratory or predictive tools (ECHA, 2017b). Few  
58 examples of toxicological endpoint assessments replaced by computational tools exist  
59 (Herrmann and Jayne, 2019).

60 Nanotechnology involves the incorporation of expertise from various disciplines such as  
61 materials science, chemistry, toxicology, computational sciences and decision-making. Current  
62 interest in Integrated Approaches to Testing and Assessment (IATA), aiming at reducing animal  
63 testing, and the fundamental research for a mechanistic understanding of NPs toxicity are  
64 expected to increase reliance on computational modeling to predict properties and (eco)toxicity

65 for new nanoforms. There is a significant momentum from scientific and policy-influencing  
66 bodies globally to promote *in silico* tools as alternatives methods. In addition, an expanding  
67 knowledge base which reinforces robust modeling competences in forecasting NP's properties,  
68 exposure and hazard potential would allow materials design with maximized utility and  
69 minimized toxicity (safe-by-design) (Schwarz-Plaschg, *et al.*, 2017, Kraegeloh, *et al.*, 2018).  
70 In recent years, the European Commission has financed modelling projects to tackle the  
71 opportunities offered by modeling the toxicity and properties of NPs (Puzyn, *et al.*, 2018,  
72 Haase, 2018).

73 Over the last two decades various types of machine learning models have been developed for  
74 predicting toxicological effects of nanoforms. As the use of computational tools in  
75 nanotoxicology is increasing, this article provides an extensive up-to-date review focusing on  
76 articles implementing machine learning tools that predict a toxicological endpoint. In this  
77 review the mechanistic interpretation of models is not analyzed; the goal of the manuscript is  
78 not to critically assess the available tools but rather to provide an overview of the machine  
79 learning tools in nanotoxicology featuring their inherent biological organization. The review  
80 maps existing models built for a specific category of NPs in the space of the features of the  
81 biological agent where the outcome is observed. Forthcoming nano-risk assessment is based on  
82 the system biology (target-organ assessment/specific level of biological organization). In order  
83 to identify the hazard potential on specific exposure conditions, we provide to computational  
84 nano-toxicologists a collective visual of applied machine learning tools. To our knowledge, this  
85 is the first review that breaks down the available computational tools based on the biology  
86 system in which they were implemented. We found that a number of computational tools have  
87 been applied for metals and/or metal oxides NPs, the majority of *in silico* studies use *in vitro*  
88 data, nano-specific features are gaining momentum as classic QSARs show inadequate to  
89 predict NP toxicity; there are no clear endpoints and/or endpoints accepted by regulators; trees,

90 neural networks and regression are the most common implemented algorithms; perturbation  
 91 and quasi-QSARs are promising approaches in the field of nanotoxicology; there is a deficiency  
 92 and lack of harmonization in descriptions of experimental design inputs; there is an ongoing  
 93 effort in substituting sparse, inhomogeneous literature data, the current common data source,  
 94 with central, curated, comprehensive databases.

### 95 3 Materials and Methods

#### 96 *Search design*

97 In order to investigate machine learning models in nanotoxicology we explored multiple  
 98 sources of peer-reviewed scientific literature and reports with a systematic Boolean search of  
 99 key terms (“nanoparticle”, “nanomaterial”, “in silico”, “computational”, “machine  
 100 learning”, “model”) to produce specific multiple search strings. Those texts have been applied  
 101 to discover studies that implement a machine learning model to predict nanotoxicity in publicly  
 102 available electronic search engines (*ScienceDirect, Web of Science, Google Scholar and*  
 103 *PubMed*) (**Table 1**). The final technical report of *NanoComput* project, “*Evaluation of the*  
 104 *availability and applicability of computational approaches in the safety assessment of*  
 105 *nanomaterials*”, carried out by the European Commission Joint Research Centre (JRC) was  
 106 taken into consideration for studies before 2017 (Worth A., 2017).

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108 **Table 1. Review protocol.**

Subject	Description	Subject	Description
Databases	<i>Google Scholar, Elsevier</i> <i>(Scopus and</i>	Exclusion criteria	Studies predicting nano- properties, environmental

	<i>ScienceDirect), Web of Science and PubMed</i>		outcomes, pharmacokinetic modelling
<b>Keywords</b>	<i>nanoparticle, nanomaterial, in silico, computational, machine learning, model</i>	<b>Publication type</b>	Peer-reviewed journals and reports
<b>Search files</b>	title, abstract, keywords	<b>Time interval</b>	Last decade

### 109 *Eligibility and exclusion criteria*

110 We focused on machine learning models predicting ecotoxicological (e.g., aquatic and  
111 terrestrial organism toxicity) and human health toxicological endpoints. In this review the  
112 endpoint is defined as: *a specific biological effect defined in terms of biological target structure  
113 and associated changes in tissue structures and/or other parameters* (OECD, 2009). Therefore,  
114 studies predicting properties of NPs such as solubility, dispersion, absorption, zeta potential,  
115 partition coefficients, Poisson's ration or Young's Modulus and environmental outcomes (e.g.,  
116 bioaccumulation, degradation) were not included. The literature review utilized different  
117 inclusion criteria for studies that i) focus on the model implementation, ii) have been published  
118 during the last decade, iii) are published in English, iv) are published in peer-reviewed journals.  
119 The search was performed through title, abstract and keywords and a cursory read to evaluate  
120 the paper relevance. Furthermore, manual searches were conducted in reference lists from  
121 published literature to discover studies that might have been overlooked by the online searches.  
122 The search resulted in 86 studies implementing machine learning models for nanotoxicity  
123 prediction, published in the last decade.

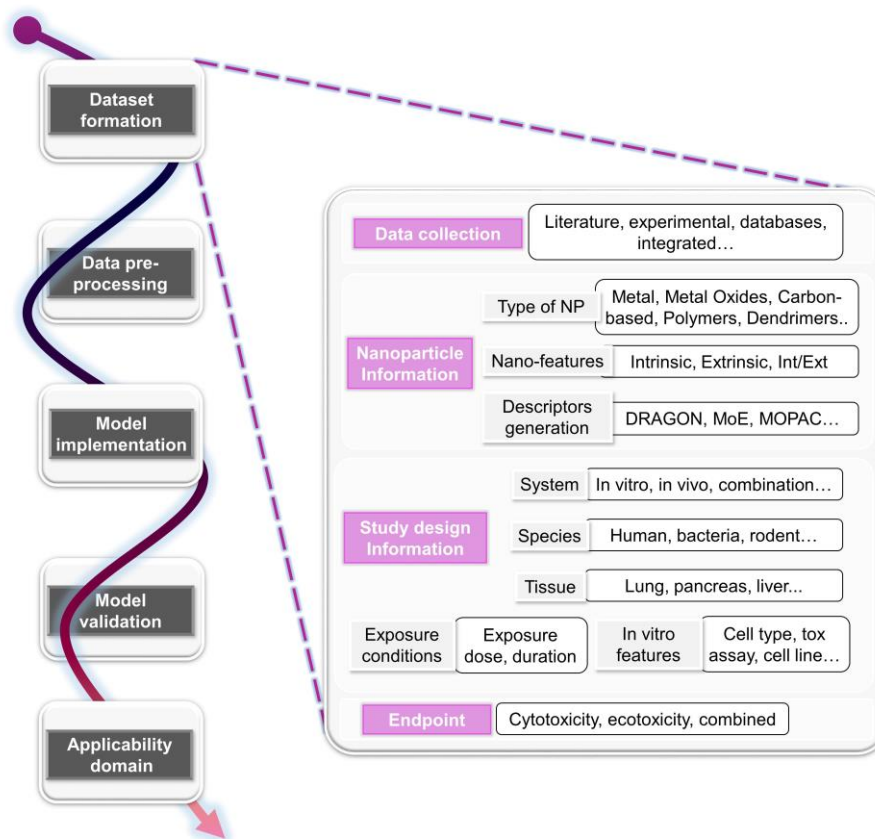
124 Computational predictive models for absorption, distribution, metabolism and excretion rely on  
125 physicochemical data and on mechanistic descriptions of the underlying biophysical and  
126 biochemical processes. Physiologically Based Pharmacokinetic (PBPK) modelling was not

127 addressed in this study as it has been addressed lately elsewhere (Li, *et al.*, 2017, Yuan, *et al.*,  
128 2019). Different schemes are proposed for grouping NPs and reviewed elsewhere (Giusti, *et*  
129 *al.*, 2019, Lamon, *et al.*, 2018, Lamon, *et al.*, 2018b). Lamon, *et al.* (2018b) reviewed published  
130 categorization schemes, grouping for read-across approaches and computational ranking of  
131 NPs. The authors stated that the limited studies that address NP similarities were based on  
132 limited datasets and the tools are not user-friendly. The authors suggested that datasets of  
133 toxicity and nano-properties should be investigated to distinguish groups of NPs. Giusti, *et al.*  
134 (2019) stated how computational methods e.g., for developing or endorsing initial grouping  
135 hypotheses, are usefully applied to different stages of grouping. A number of techniques can be  
136 used to predict toxicity, ranging from read-across, unsupervised and supervised methods of  
137 machine learning, to a variety of QSAR methods.

### 138 ***Information extraction***

139 The relative toxicity of NPs can be determined by numerous factors such as the route of  
140 exposure, dose and duration, as well as the p-chem properties. Furthermore, the experimental  
141 parameters i.e., the cell or animal model, cell line, cell type (normal or cancer), toxicological  
142 assay (MTS, MTT, etc., for *in vitro*) or the gender and age for *in vivo* assays, add further  
143 variables that may affect the outcome. In this study, each paper was reviewed and information  
144 related to the i) data extraction source (literature, database or experimental), ii) NPs category  
145 (metal, metal oxides et.), iii) nano-specific descriptors (p-chem), iv) study design experimental  
146 parameters (*in vitro/in vivo*, dose, cell line, tissue, etc.), v) endpoints and vi) model algorithms  
147 (neural networks, regression etc.) were extracted . The proposed work is the review of the above  
148 information on the existing studies and the representation in an integrated style. There are no  
149 definite guidelines in dataset formation and model implementation in order to create a  
150 predictive model with nanotoxicological data. A figure with all the steps, from data extraction  
151 to model validation and applicability domain is provided below. The steps shown serve as a

152 guide for extracting and categorizing the information of the published studies in a systematic  
 153 way and were adopted in this review.



154

155 **Figure 1. A summarized general roadmap for implementing a model in the field of**  
 156 **nanotoxicology.** The roadmap can be divided into five main parts: dataset formation overview,  
 157 data pre-processing, model implementation, model validation and applicability domain.

158

159 Following the general roadmap of model implementation shown in **Figure 1**, we discriminated  
 160 five main sequential parts involved. The first step, the dataset formation, where  
 161 nanotoxicological data is gathered to be used by predictive models. Sources, features of NPs,  
 162 study design information and patterns of data used in the studies during the last decade were  
 163 reviewed and mapped towards the five most common predicted endpoints.

164 The remaining sequential parts of **Figure 1** i.e., data pre-processing techniques, model  
 165 implementation, model validation and model applicability domain were covered in greater

166 detail elsewhere (Furxhi, *et al.*, 2020b). The Dataset formation overview contains four subparts  
167 (**Figure 1**). The first part refers to the data sources used in the studies being either existing  
168 literature, databases or new data experimentally created or their combinations. In the second  
169 part the information on NPs is extracted e.g., nano-specific descriptors (size, coating, zeta  
170 potential, etc.) and the NP category (metal, metal oxide, carbon based, etc.). Besides nano-  
171 specific descriptors, theoretical descriptors can be generated using available software to be used  
172 as input. Third, inputs including study design information are attained such as the testing system  
173 (*in vitro*, *in vivo*), species (human, bacteria, etc.), tissue (lung, kidney, etc.), exposure conditions  
174 (dose, duration) and *in vitro* experimental features (e.g., cell line: A549, Caco2, etc.). Last, the  
175 toxicological studies endpoints which are used as the output to be predicted by the models are  
176 recorded. All of the reviewed studies mentioned data sources, NP category, endpoint predicted  
177 and model algorithm chosen. In cases where the study did not mention the biological features  
178 of the experimentally measured endpoint, we tracked down the original publication of the data  
179 to find out the toxicological assay information.

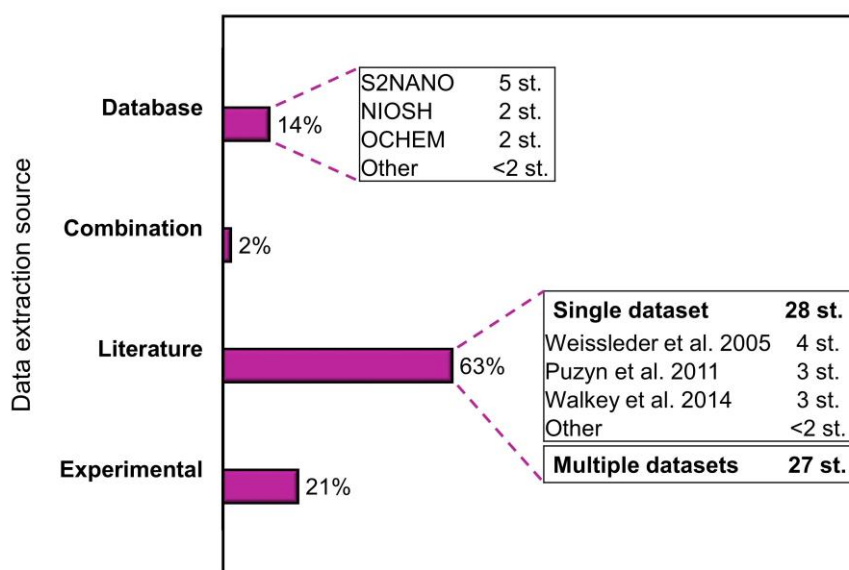
## 180 **4 Results**

### 181 **4.1 Dataset formation overview**

#### 182 4.1.1 *Data sources in the studies*

183 The majority of the reviewed studies (63%) extracted data from peer-reviewed literature  
184 sources (**Figure 2**). A significant portion, 21%, of the studies, used new data derived  
185 experimentally either from NP synthesis and elaborated characterization or *in vitro/in vivo*  
186 toxicological assays. 14% of the studies exploited available datasets from nano-specific or non-  
187 nanospecific databases. In contrast, only 2% of the studies used integrated data combining  
188 literature, database and experimentally produced new data. A notable share of publications  
189 applied extensively a few datasets (Lamon, *et al.*, 2019). In detail, 28 studies that extracted a

190 single dataset from the literature (see **Figure 2- Literature**) used mostly data originally from  
 191 (Weissleder, *et al.*, 2005, Puzyn, *et al.*, 2011, Walkey, *et al.*, 2014) for the prediction of either  
 192 the bacterial viability of metal oxides or the prediction of cellular uptake of florescent magnetic  
 193 metal oxides. The rest of the studies handled multiple datasets extracted from different peer-  
 194 reviewed journals. Safe and Sustainable Nanotechnology (S2NANO)<sup>1</sup> was the database mostly  
 195 used (see **Figure 2- Database**); other databases such as the National Institute for Occupational  
 196 Safety and Health (NIOSH)<sup>2</sup>, the Online chemical database (OCHEM)<sup>3</sup>, the Database and  
 197 Ontology Framework for Nanomaterials Design and Safety Assessment (eNanoMapper)<sup>4</sup> and  
 198 Nanomaterial-Biological Interactions Knowledgebase (NBI)<sup>5</sup> were used less.



199

<sup>1</sup> <http://portal.s2nano.org/> (Webpage accessed autumn 2019).

<sup>2</sup> <https://www.cdc.gov/niosh/data/default.html> (Webpage accessed autumn 2019).

<sup>3</sup> <https://ochem.eu/home/show.do> (Webpage accessed autumn 2019).

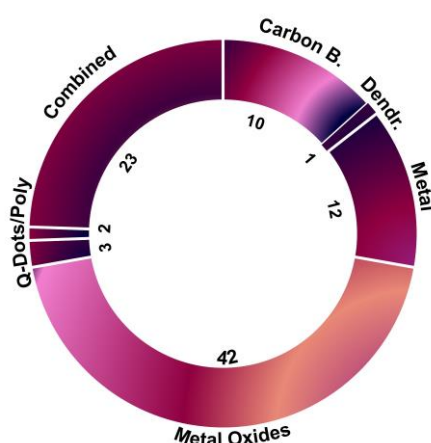
<sup>4</sup> <http://www.enanomapper.net/data> (Webpage accessed autumn 2019).

<sup>5</sup> <http://nbi.oregonstate.edu/> (Webpage accessed autumn 2019).

200 **Figure 2. The percentage of data extraction sources and experimentally generated new**  
 201 **data from the gathered studies.** Additional information on the number of studies in case of  
 202 literature and database data extraction is shown.

#### 203 4.1.2 Nanoparticle and study design information

204 Different categories of NPs based on chemical composition were studied during the last  
 205 decade regarding their toxicity prediction using machine learning models. The data in **Figure**  
 206 **3** indicate that metal oxides (i.e., Al<sub>2</sub>O<sub>3</sub>, CuO, TiO<sub>2</sub>, CeO<sub>2</sub>, La<sub>2</sub>O<sub>3</sub>, Co<sub>3</sub>O<sub>4</sub>, Cr<sub>2</sub>O<sub>3</sub>, Sb<sub>2</sub>O<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub>,  
 207 Fe<sub>3</sub>O<sub>4</sub>, SnO<sub>2</sub>, Ni<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub>, Mn<sub>2</sub>O<sub>3</sub>, ZnO etc.,) were the most abundantly modelled NPs (42  
 208 studies) followed by carbon based (nano-tubes and fullerenes) and metals (Au, Ag, Cu, Co,  
 209 etc.,) with 10 and 12 studies, respectively. A limited number of studies dealt with dendrimers  
 210 such as Poly-(amido amine) (Jones, *et al.*, 2015), polymers (chitosan/streptokinase, Poly(N-  
 211 isopropylacrylamide)) (Bygd, *et al.*, 2015, Baharifar and Amani, 2016) and quantum dots  
 212 (Bilal, *et al.*, 2019, Oh, *et al.*, 2016). The above studies used datasets specific to one NP  
 213 category; nonetheless, different categories of NPs such as metals, metal-oxides, carbon-based  
 214 NPs etc., were commonly merged in one dataset for model implementation (23 studies).



215

216 **Figure 3. Nanoparticles categories in the datasets used for modelling in the studies**  
217 **gathered.** The numbers demonstrate the number of studies using the specific category. The sum  
218 is higher than sum of studies gathered as some studies dealt with more than one dataset.

219 P-chem properties can be generally categorized into intrinsic properties (core size, shape,  
220 chemical composition) related to the NP itself, extrinsic (zeta potential, purity, agglomeration)  
221 that is, properties related to the behavior of NP in the exposure media, and intermediate  
222 properties (coating, surface area, solubility) (Casals, *et al.*, 2017). Almost half of the studies  
223 used at least one nano-specific descriptor as input. Size (measured in different media using  
224 different techniques) showed up most times (38 studies out of 86) which is evident in **Figure 4**  
225 since it is a common feature that has been linked to nanotoxicity. Size was followed by zeta  
226 potential and surface area given in 30 and 16 studies, respectively. Coating, shape and  
227 composition appeared as significant features in modelling, whereas agglomeration, solubility,  
228 purity and composition were not considered as much presumably due to the lack of data.  
229 Exposure conditions such as dose and exposure time were also monitored with dose appearing  
230 more frequently (27 studies). The bottom-right corner cell in **Figure 4** demonstrates studies that  
231 took at least one *in vitro* characteristic such as cell line, cell origin, assay, cell type and species,  
232 as final input descriptor in the model. Studies typically used a combination of the  
233 aforementioned variables and selected the final descriptors for modelling after pre-processing  
234 the data and eliminating the least important features. Data pre-processing techniques and feature  
235 selection methods used in the studies are covered in greater detail elsewhere (Furxhi, *et al.*,  
236 2020b). Protein-corona descriptors (*not shown in figure*) were used in two studies (Papa, *et al.*,  
237 2016, Liu, *et al.*, 2015).



238

239 **Figure 4. Nano-specific features used in the studies gathered.** The number signifies the  
 240 number of studies (out of 86) using that parameter as input in the final model.

241 *In vivo* features in the *study design information* were not placed in the first section of **Figure 1**,  
 242 since only a limited amount of studies used experimental *in vivo* information as input variables.

243 Ten out of 86 studies referred to an *in vivo* system (rodent, fish, crustacean or combination of  
 244 organisms); nevertheless, three of them utilized *in vivo* experimental information as final  
 245 descriptors. Ban, *et al.* (2018) used the animal strain, weight, exposure route and age of rodents  
 246 for the prediction of reproductive toxicity using a decision tree algorithm (random forest).  
 247 Gernand and Casman (2014) utilized the animal weight for a meta-analysis prediction of carbon  
 248 based NPs for four different endpoints using random forests. Liu, *et al.* (2013) exploited the  
 249 primary exposure route to predict the post-fertilization mortality, translated in a metric based  
 250 score for zebrafish. However, while other studies took a combination of *in vitro* - *in vivo* studies  
 251 (Kleandrova, *et al.*, 2014b, Kovalishyn, *et al.*, 2018), *in vivo* assays have not been favored in  
 252 nanotoxicity predictions verifying the picture of the field landscape described in (Basei, *et al.*,  
 253 2019).

#### 254 4.1.3 Descriptors generation in the studies

255 Quantitative Structure-Activity Relationships (QSARs) models are based on the  
256 mathematical descriptions of the relationship between molecular structural characteristics and  
257 an effect (Christen, *et al.*, 2014, Sizochenko, *et al.*, 2014). Normally, the structure of NPs can  
258 be defined by both experimentally determined p-chem properties and theoretically calculated  
259 descriptors. From the 86 studies gathered half of them calculated theoretical descriptors as input  
260 variables either used as such, or in combination with nano-specific characteristics. Theoretical  
261 descriptors can be calculated using academic and commercial software (e.g., ADRIANA<sup>6</sup>,  
262 Dragon<sup>7</sup>, Cerius2<sup>8</sup>, MOE<sup>9</sup>, MOPAC<sup>10</sup>, PaDEL<sup>11</sup> etc.) derived from NP electrical, atomic and  
263 molecular structure and their immediate environment (Gharagheizi and Alamdari, 2008,  
264 Gerber, *et al.*, 2013, Petrova, *et al.*, 2011). However, porting the complex and non-uniform  
265 nanostructures in computer language is a challenging and time-consuming task (Oksel, *et al.*,  
266 2017). Density Functional Theory (DFT) as an instance of determining molecular descriptors  
267 is a method that settles among quick ad hoc, semi-empirical and time-intensive ab initio  
268 methods (Villaverde, *et al.*, 2018, Pathakoti, *et al.*, 2014).

269 Quantum chemical descriptors were calculated in 18 out of 86 studies. For the ones used the  
270 most the calculations require certain quantum-chemical background (Nikota, *et al.*, 2016).  
271 Electron distribution and affinity, band gap, electronegativity and enthalpy of formation are  
272 commonly used descriptors derived from quantum-mechanical calculations. A new approach,  
273 the Liquid Drop Model (LDM) combines specific descriptors that represent a NP  
274 supramolecular structure for different organizational levels avoiding extensive quantum-

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<sup>6</sup> <https://www.mn-am.com/products/adrianacode>. (Webpage accessed autumn 2019).

<sup>7</sup> [https://chm.kode-solutions.net/products\\_dragon.php](https://chm.kode-solutions.net/products_dragon.php). (Webpage accessed autumn 2019).

<sup>8</sup> <http://www-jmg.ch.cam.ac.uk/cil/SGTL/cerius2.html>. (Webpage accessed autumn 2019).

<sup>9</sup> <https://www.chemcomp.com/>. (Webpage accessed autumn 2019).

<sup>10</sup> <http://openmopac.net/>. (Webpage accessed autumn 2019).

<sup>11</sup> <http://padel.nus.edu.sg/software/padeldescriptor/>. (Webpage accessed autumn 2019).

275 mechanical calculations (Sizochenko, *et al.*, 2014). The OECD validation principles require  
276 that the exact process, followed to obtain extract descriptors, should be explicitly stated (Puzyn,  
277 *et al.*, 2018).

278 Descriptors to characterize NPs may be taken directly from the Chemicool<sup>12</sup> periodic table  
279 (Jagiello, *et al.*, 2016, Kleandrova, *et al.*, 2014). Periodic table descriptors were used in nine  
280 studies out of 86 studies. An alternative source are images taken by transmission electron  
281 microscopy (TEM), scanning electron microscopy or atomic force microscopy (Bigdeli, *et al.*,  
282 2015). Using images derived from TEM, quantum-mechanical and nano-specific descriptors  
283 such as shape, size, aggregation state and surface area can be generated (Jagiello, *et al.*, 2016,  
284 Bigdeli, *et al.*, 2015, Mikolajczyk, *et al.*, 2015). Experimental descriptors can also be obtained  
285 from Raman spectroscopy (González-Durruthy, *et al.*, 2017).

#### 286 4.1.4 *Studies Endpoints*

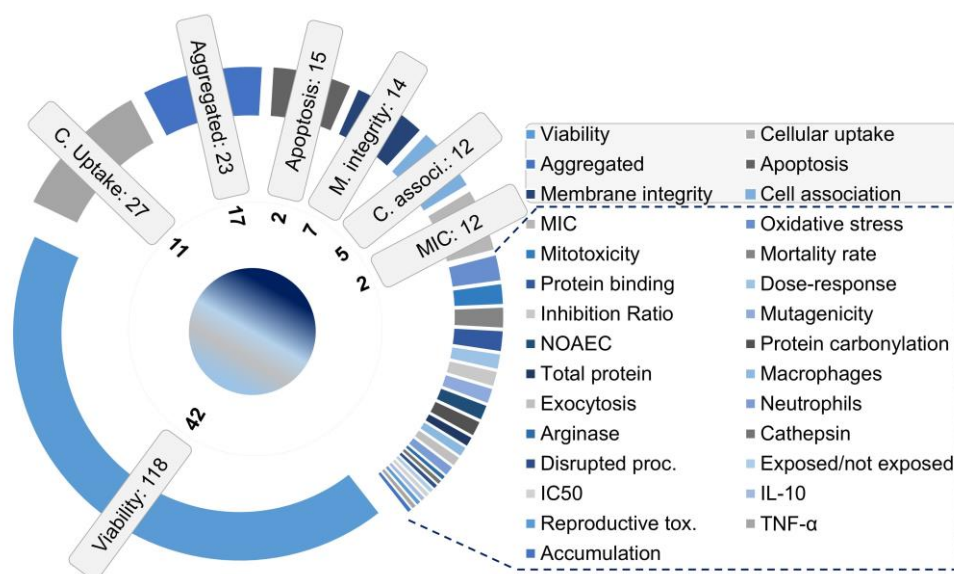
287 The first OECD principle of a QSAR and an *in silico* models in general requires a *well-*  
288 *defined endpoint*. The endpoint is defined as “*a measure of activity for chemicals made under*  
289 *specific conditions*” and refers to “*any physicochemical property, biological effect or*  
290 *environmental parameter related to chemical structure that can be measured and modelled*”  
291 (OECD, 2004). In the studies gathered the predicted endpoints were in general clearly identified  
292 and several toxicological endpoints were used. The combinations of experimental *in vitro*  
293 parameters for each of the studies, such as species, cell type and tissue, NP category, model  
294 implementation and endpoints, were recorded independently in a worksheet resulting in  
295 multiple insertions (rows) per study. If for example, a different model with unchanged  
296 conditions was created, this was inserted as a novel case (row) within the worksheet.  
297 Alternatively, if different data-rows were used by the same model this leads to a new case in

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<sup>12</sup> <https://www.chemicool.com/>. (Webpage accessed autumn 2019).

298 our analysis. This process resulted in the extraction of 273 predictive modelling cases  
299 implemented in 86 individual studies. All the cases are derived from the studies. A broad  
300 spectrum of *in vitro* assays are currently used since they are important indicators for biological  
301 evaluation. *In vitro* assays are cheap, rapid and reproducible covering various cell functions.  
302 Assays measure different markers, signaling the loss of membrane integrity (cytotoxicity), the  
303 number of live cells (viability assay) or cell death mechanisms (e.g., apoptosis).

304 From the 273 cases the majority (118) was built to predict *viability* (**Figure 5**). These 118 cases  
305 were extracted from 42 individual studies. There are numerous classifications of cell viability  
306 assays, labeled as e.g., colorimetric, dye exclusion, fluorometric assays etc. In this review, we  
307 generally cited the endpoints without splitting them into further detailed assays for reasons of  
308 simplicity and clarity. The second most predicted endpoint was *cellular uptake*. 27 cases to  
309 predict cellular uptake were derived from 11 studies. *Cellular uptake* relate directly to the  
310 processes leading NPs to penetrate the cell membrane (Zhao, *et al.*, 2011) and facilitates  
311 understanding both adverse (toxicological) and favorable (i.e., drug delivery) biological fate of  
312 NPs. *Aggregated* outcomes were predicted in 23 cases derived from 17 studies. The term  
313 “aggregated” refers to a combination of different endpoints in predicting a generic biological  
314 activity. Usually those studies performed weighting averages of the combined outcomes or  
315 ranking of hazard endpoints into a singular outcome. *Apoptosis* (the process of programmed  
316 cell death) was targeted in 15 cases derived from 2 studies and *membrane integrity* in 14 cases  
317 produced by 7 studies. Membrane integrity is defined as the quality or state of the complete cell  
318 membrane in perfect condition (Elmore, 2007).



319

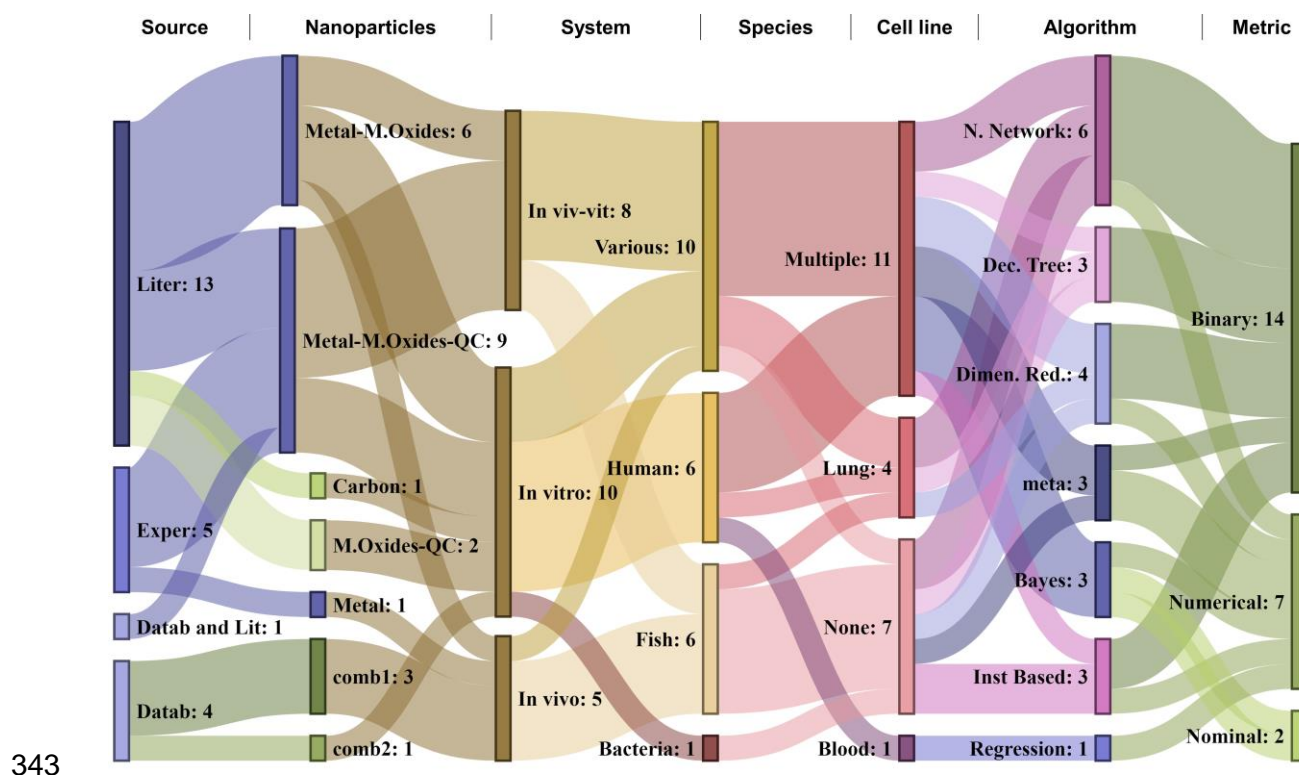
320 **Figure 5. Outcomes-nanotoxicological endpoints that are predicted in the field of**  
 321 **computational nanotoxicology.** The value next to the outcome, inside the box, is the number  
 322 of model implementations (cases) performed to predict that outcome. The number outside the  
 323 box (bold) is the number of individual studies that implemented the computational tools.

324 The rest of the outcomes (**Figure 5, box surrounded with dash type lines**) such as oxidative  
 325 stress of metal oxides (Gajewicz, *et al.*, 2018), mitotoxicity (mitochondrial respiration) of  
 326 carbon nano-tubes (González-Durruthy, *et al.*, 2017), exocytosis of gold NPs (Bigdeli, *et al.*,  
 327 2015, Oksel, *et al.*, 2016), reproductive toxicity of various NPs (Ban, *et al.*, 2018), macrophages  
 328 or neutrophils counts as pulmonary toxicity indicators (Gernand and Casman, 2014),  
 329 mutagenicity of carbon-based NPs (Toropov and Toropova, 2014, Toropov and Toropova,  
 330 2015) etc., were targeted in less than four cases resulting from few studies. We performed a  
 331 focused analysis of the top five most common nanotoxicological endpoints (viability, cellular  
 332 uptake, aggregated outcomes, apoptosis and membrane integrity).

## 333 4.2 Case mapping by endpoint overview

334 In the section below, information gathered from the 273 cases was further analyzed to  
 335 reveal pathways through data extraction source, NP groups, level of biological organization  
 336 (system, species, tissue), model implementation and endpoint metrics that lead to the top-five  
 337 toxicological predicted endpoints. The review is split and presented by endpoint.

338 The most common data mining machine learning algorithms can be clustered to categories such  
 339 as *rules*, *instance based* (Inst Based), *decision trees* (D. Tree), *bayesian networks* (Bayes),  
 340 *neural networks* (N. Network), *dimensionality reduction* (D. reduction), *regression* and  
 341 *meta/ensemble* (Brownlee, 2013). Furxhi, *et al.* (2020b) gave a detailed information of the  
 342 machine learning algorithms for the 273 cases.



344 **Figure 6. Case flow by attribute, featuring aggregated endpoints as a combined outcome.** The  
 345 width of the flows is proportional to the number of cases. The flow demonstrates what cell lines of which  
 346 species were exposed to which particles, in *in vivo* or *in vitro* experiments, resulting in various endpoints  
 347 aggregated to one outcome. It also shows what models were used for predicting the aggregated outcome,

348 codified as either binary, numerical or nominal. *Liter: literature, Exper: experimental, QC: quantum-dots,*  
 349 *comb1: dataset containing metal, metal oxides, polymeric and dendrimer NPs, comb2: dataset containing*  
 350 *metal, metal oxides and carbon based NPs, in viv-vit: dataset containing in vivo and in vitro data*

351 The Sankey diagram in **Figure 6** shows the information flow of data starting from their  
 352 extraction source through their NP types, experimental biological system and tissue studied,  
 353 algorithm implemented and outcome metrics used to predict *aggregated* outcomes (**Figure 6**).  
 354 *Aggregated* outcomes correspond to the combination of multiple endpoints under certain  
 355 criteria or the combination of endpoints with experimental conditions and/or toxicities/activities  
 356 (**Table 2**). *Aggregated* outcomes were mostly predicted by models using data extracted from  
 357 the literature (13 cases) followed by experimental new data and databases, 5 and 4 cases,  
 358 respectively (**Figure 6**). The largest portion of the models used datasets containing metals-metal  
 359 oxides-quantum dots and metals-metal oxides, 9 and 6 cases, respectively. Over a half of the  
 360 models predicted *aggregated* outcomes from *in vitro* (10 cases) systems using various species  
 361 (10 cases) with multiple cell types (11 cases) (**Table 2**).

362 **Table 2. Terms referring to grouped values in the review:** Aggregated (outcomes), NPs  
 363 (Comb), species (various in **Figure 6**), cell lines (multiple in **Figure 6**) and algorithm (Meta in  
 364 **Figure 6, Figure 8 and Figure 10**) examples.

<b>Aggregated (outcomes)</b>	Caspase-mediated apoptosis, mitochondrial membrane potential, mitochondrial depolarization, neurological, pulmonary, fibrosis, immunological, genotoxicity, inflammation, particle concentration in air and occupational exposure limits, Micro-Eff, membrane integrity, necrosis, haemolysis, mitochondrial function, CC <sub>50</sub> , EC <sub>50</sub> , IC <sub>50</sub> , TC <sub>50</sub> , LC <sub>50</sub> , MIC, MBC among others combined.
<b>Various (species)</b>	Algae, bacteria, fungi, mammal, human, crustaceans, plants, fishes, protozoa, plants, amphibians, nematodes among others combined.

<b>Multiple (cell lines or species details)</b>	Danio rerio, Pseudokirchneriella subcapitata, aorta, coronary artery, monocytes/ macrophage, liver, skin, colon, Escherichia coli, Photobacterium phosphoreum, Vibrio fischeri, Saccharomyces cerevisiae, Desmodesmus subspicatus, Chlorella vulgaris, Scenedesmus, Brassica napus, Cucumis sativus, Raphanus sativus, Lolium perenne, Daphnia magna, Thamnocephalus platyurus, Ceriodaphnia dubia, Poecilia reticulata, among others combined.
<b>NPs (Combina- tions)</b>	Comb1: metal, metal oxides, polymers and dendrimers in one dataset Comb2: metal, metal oxides and carbon based NPs in one dataset.
<b>Algorithm (meta)</b>	Bagging algorithm, Stochastic gradient boosting and majority voting. Combination of several different individual classifiers.

365

366 A paradigm of perturbation models that use multiple species and cell lines to predict an  
367 aggregated outcome can be found in Concu, *et al.* (2017) who predicted the toxicity profile of  
368 metals-metal oxides-quantum dots NPs based on several measures such as the concentration  
369 causing i) 50% reduction in viability ( $CC_{50}$ ), ii) a response halfway between the baseline and  
370 maximum ( $EC_{50}$ ), iii) prevention of the root elongation of a plant at 50% ( $IC_{50}$ ), iv) toxic ( $TC_{50}$ )  
371 and v) lethal ( $LC_{50}$ ) effects. The authors developed a Quantitative Structure–Toxicity  
372 Relationship (QSTR) perturbation model based on data for mammal cell lines, algae, fish,  
373 bacteria and others. Using perturbation theory and moving average analysis they defined the  
374 final descriptors based on pair-combinations differences. The outcome was clustered into a  
375 binary metric form the so called “toxic” and ‘non-toxic’. The same approach was followed in  
376 (Kleandrova, *et al.*, 2014, Kleandrova, *et al.*, 2017, Luan, *et al.*, 2014). As clearly shown in  
377 **Figure 6**, the majority of the models predicting *aggregated* outcomes used binary metrics (14  
378 cases).

379 *In silico* tools for *in vivo* systems (4 cases) were used for cases of fish organisms. George, *et al.*  
380 (2011) studied commercial metals/metal oxides with the inclusion of a quantum dot, using a  
381 multi-parametric screening assay incorporating cellular injuries (ROS production, intracellular  
382 calcium flux, mitochondrial depolarization, and plasma membrane permeability). The authors  
383 applied *neural networks* to estimate and compare the *in vitro* hazard potential. The zebrafish  
384 was used as an *in vivo* model to develop the hazard ranking for NPs based on the impact on  
385 zebrafish embryos.

386 Speck-Planche, *et al.* (2015) used data from literature and the OCHEM database for the  
387 prediction of a binary ecotoxicological outcome using a QSAR-perturbation model based on  
388 the concentration for 50% inhibition of growth (IC<sub>50</sub>), minimum concentration preventing the  
389 visible growth (MIC) or completely killing of the bacteria (MBC) and the microbicidal effect  
390 under several experimental conditions. Available databases were also used; the NBI knowledge  
391 base was used by (Liu, *et al.*, 2013) for the prediction of post-fertilization mortality of zebrafish  
392 translated into a metric based score for metal, dendrimer, metal oxide and polymeric NPs  
393 (*Comb1* in **Figure 6**); the NIOSH database combined with an EU expert consortium was used  
394 by (Murphy, *et al.*, 2016) for the risk estimation of carbon-based, metal and metal oxide NPs  
395 (*Comb2* in **Figure 6**). The authors used a *Bayesian Network* in a control banding framework.

396 Metals-metal oxides datasets were used to create 6 models predicting *aggregated* outcomes  
397 using various species and multiple cell lines. Oksel, *et al.* (2016) described the application of a  
398 *decision tree* algorithm for nano-Structure–Activity Relationship (SAR) modeling using four  
399 different paradigms. The first paradigm was a dataset for the general cellular toxicity in a binary  
400 form for oxidative stress and acute pulmonary inflammation based on multi-parameter high-  
401 throughput screening assays.

402 Two cases predicted an *aggregated* outcome in a nominal form, as seen in **Figure 6**. Marvin,  
403 *et al.* (2017) used a *Bayesian* model to rank human hazard of metals-metal oxides, into low,  
404 medium, high and none (nominal metrics), based on several biological effects such as  
405 neurological, immunological, inflammation, fibrosis etc. Harper, *et al.* (2015) summarized 21  
406 measured ecotoxicological endpoints from zebrafish to develop an a priori weighting metric  
407 score for effect comparison implementing a *decision tree* algorithm. Shao, *et al.*, 2013 applied  
408 a *regression* case for carbon nanotubes using several endpoints based on an activity scale to  
409 predict human toxicity in blood cells (Shao, *et al.*, 2013).

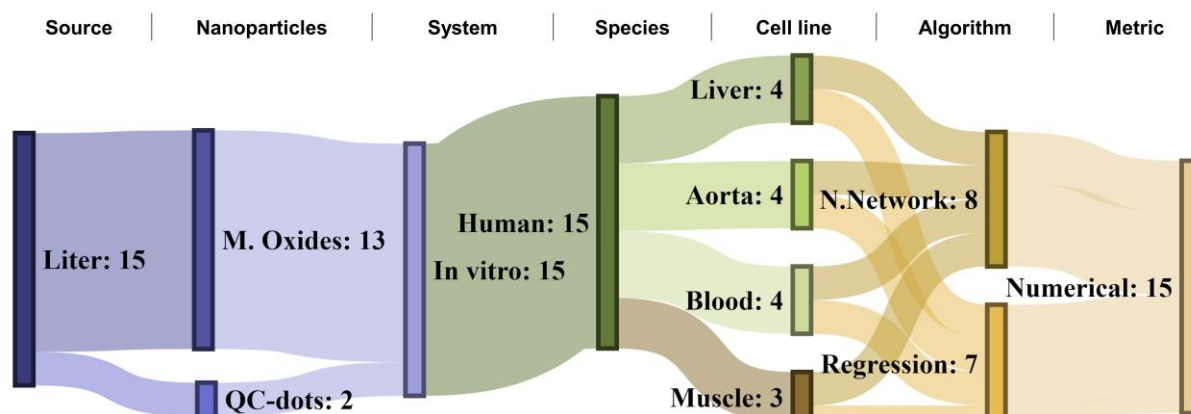
410 Sizochenko, *et al.*, 2018 developed a unsupervised neural network to predict the toxicity of  
411 metal oxides regarding bacteria, algae, protozoa and mammalian cell lines. Oxidative stress and  
412 acute pulmonary inflammation were used as endpoints in (Zhang, *et al.*, 2012) to demonstrate  
413 the effect of band gap and dissolution profiles on metal oxides toxicity using a regression tree.  
414 Fourches, *et al.* (2010) used an *instance based* algorithm to predict metal NPs impact on ATP  
415 content, reducing equivalents, caspase-mediated apoptosis and mitochondrial membrane in one  
416 dataset using biological input of dose, cell line, and assay in a combinatorial vector.

417 In summary, *aggregated* outcomes were usually predicted by data extracted from the literature,  
418 mostly for metals, metal oxides and quantum dots, tested *in vitro* for various species and  
419 multiple cell lines thereby building up large datasets. Due to the complexity and magnitude of  
420 the data that refers to different species and aggregated outcomes, non-linear modelling was  
421 preferred, mostly *neural networks* and *dimensionality reduction* algorithms. **Table 3** presents  
422 the studies with the largest datasets. The largest datasets were generated using perturbation  
423 theory. Usually the datasets in nanotoxicology are small (67% of cases had less than 200 data  
424 rows).

425 **Table 3. Examples of cases that used large complex datasets.** The row of the datasets and  
 426 their accessibility.

Reference	Algorithm	Data rows	Data accessibility
(Concu, <i>et al.</i> , 2017)	<i>Neural</i>	54,371	Data available as supplementary material
(Sizochenko, <i>et al.</i> , 2018)	<i>Networks</i>	184	
(George, <i>et al.</i> , 2011)		4,032	
(Speck-Planche, <i>et al.</i> , 2015)	<i>Dimen.</i>	69,231	-
(Kleandrova, <i>et al.</i> , 2014)	<i>Red.</i>	5,520	Data available as supplementary material
(Kleandrova, <i>et al.</i> , 2014b)		36,488	

427

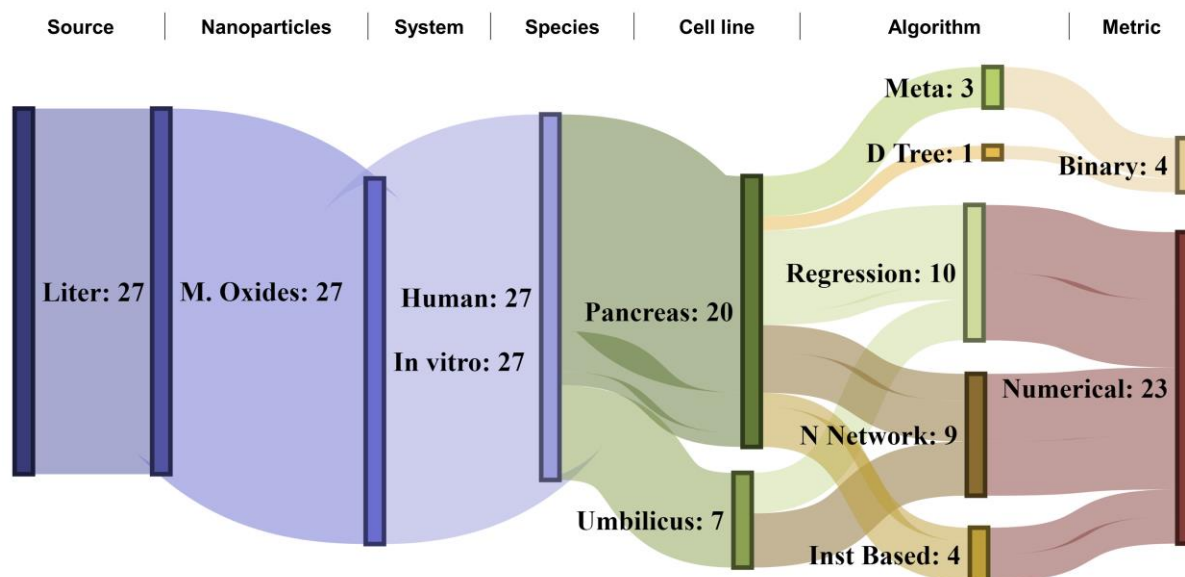


428

429 **Figure 7. Case flow by attribute, featuring apoptosis as outcome.** The width of the flows is  
 430 proportional to the number of cases. The flow demonstrates what cell lines of which species  
 431 were exposed to which particles, in *in vivo* or *in vitro* experiments, resulting in cell apoptosis.  
 432 It also shows what models were used for predicting apoptosis, codified as either a binary,  
 433 numerical or nominal outcome (only numerical in this case). *Liter*: literature, *QC-dots*: quantum-  
 434 dots.

435 The Sankey diagram in **Figure 7** shows the information flow for *apoptosis* outcome. All  
 436 datasets are derived from the literature and refer to metal oxides and quantum dots. All the

437 models were based on the apoptosis measured in human cell lines of different organ tissues,  
 438 such as liver, aorta, blood and muscle. The algorithms used were *neural networks* and  
 439 *regression* modelling of the dose-response curve (Hill slopes of EC<sub>50</sub>). In detail, Winkler, *et al.*  
 440 (2014) generated a *regression* and a Bayesian-regularized *neural network* model, using four  
 441 nanospecific descriptors that predict smooth muscle apoptosis induced by 50 metal oxides. The  
 442 authors observed that the most influential features on prediction were the core material, coating  
 443 and surface charge. Epa, *et al.* (2012) employed both linear and non-linear neural networks.  
 444 Using all p-chem and theoretical descriptors they extracted the best models and using only  
 445 interpretable descriptors, mainly size/shape of molecules and hydrogen bonding, they built two  
 446 additional models. Both studies extracted data of metal oxides in endothelial and smooth muscle  
 447 cells, monocytes and hepatocytes using four assays at four different concentrations per assay.  
 448 In both studies only QSAR-models in smooth muscle cell apoptosis assay had results of  
 449 statistical significance. Both studies took the original data from (Shaw, *et al.*, 2008).



450  
 451 **Figure 8. Case flow by attribute, featuring cellular uptake as outcome.** The width of the  
 452 flows is proportional to the number of cases. The flow demonstrates what cell lines of which  
 453 species were exposed to which particles, in *in vivo* or *in vitro* experiments, resulting in cellular

454 uptake. It also shows what models were used for predicting cellular uptake, codified as either a  
455 binary or numerical outcome. *Liter: literature.*

456 As seen in **Figure 8** all the cases predicting *cellular uptake* extracted information from  
457 literature. The datasets comprised of metal oxides tested in human cells using different cell  
458 lines, mostly pancreatic cells. Models were created to predict the *cellular uptake* in a numeric  
459 form (log[NP]/cell pM, 23 cases) or in a binary form (4 cases). Linear models such as *regression*  
460 and non-linear i.e., *neural networks, instance-based, decision trees* were used. All studies used  
461 the data reported by (Weissleder, *et al.*, 2005) for 109 superparamagnetic and dextran-coated  
462 NPs. Uptake was found to vary depending on surface modifications in PaCa2 and HUVEC of  
463 the five assays tested.

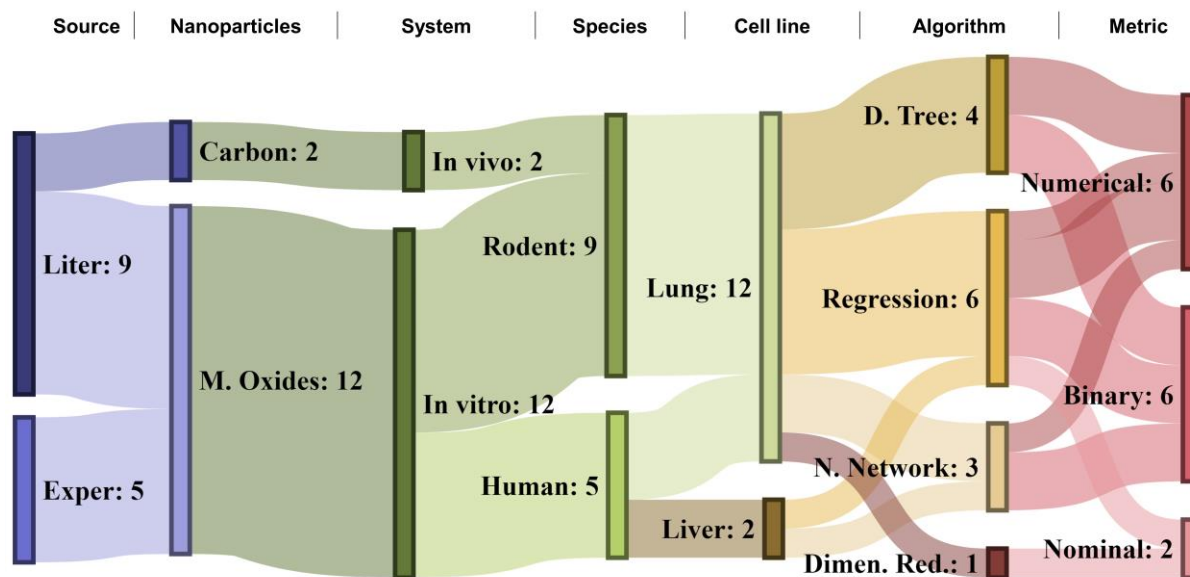
464 Fourches, *et al.* (2010) investigated the uptake against different cell lines selecting the  
465 pancreatic cells for in-depth QSAR study. Employing *instance based* (k-nearest neighbors and  
466 support vector machine) methods from the Chemical Computing Group<sup>13</sup> commercial software,  
467 using MOE descriptors, they demonstrated the importance of lipophilicity. Chau and Yap  
468 (2012) selected the above datasets based on three quality criteria such as, a sample size,  
469 endpoints not easily measured experimentally and data with standardized experimental protocol  
470 and developed a Quantitative Nanostructure Activity Relationship (QNAR) model. They built  
471 a consensus model (meta algorithm) based on *bayes* (Naive Bayes), *regression* (Logistic  
472 Regression) and *instance based* (kNN, SVM) models. The outcome of the model was in a binary  
473 form with a threshold value of 5000 NPs per cell. The same dataset was used in an ensemble  
474 QSAR model implementing stochastic gradient boosting and bagging algorithms, to predict  
475 biological effects from simple structural descriptors for a binary classification (Singh and  
476 Gupta, 2014).

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<sup>13</sup> <https://www.chemcomp.com/index.htm>. (web address accessed in summer 2019).

477 Epa, *et al.* (2012) generated *regression* and *neural network* models to predict uptake expressed  
478 as  $\log[\text{NP}]/\text{cell pM}$  using molecular descriptors from several software. Ghorbanzadeh, *et al.*  
479 (2012) also predicted the uptake using molecular descriptors calculated with Hyperchem and  
480 DRAGON software by combining *neural networks* and *regression* techniques to correlate  
481 nanostructure with bioactivity. Toropov, *et al.* (2013) predicted uptake with a quasi-QSAR  
482 model in CORAL based on SMILES-based optimal descriptors. They suggested the concept of  
483 QSARs as random events as the alternative to build up QSARs with just one distribution of  
484 available data for the training and validation subsets. Winkler, *et al.* (2014) used a regression-  
485 based QSAR model with an expectation minimization algorithm and *neural networks* with  
486 Gaussian or Laplacian prior to predict cellular uptake from theoretical descriptors.

487 Melagraki and Afantitis (2014) developed a QNAR model for the prediction of the uptake using  
488 a set of molecular descriptors encoding two-dimensional structure information. Rong, *et al.*,  
489 2015 tested linear and non-linear QSARs based on support vectors with descriptor sets selected  
490 by SFS and SFFS from an initial pool of 151 MOE descriptors for PaCa2 uptake. Finally, Oksel,  
491 *et al.* (2016) using the same dataset described the application of a novel *decision tree* algorithm  
492 for binary classification that could provide more clarity for interpreting the NP structure–  
493 activity models.



494

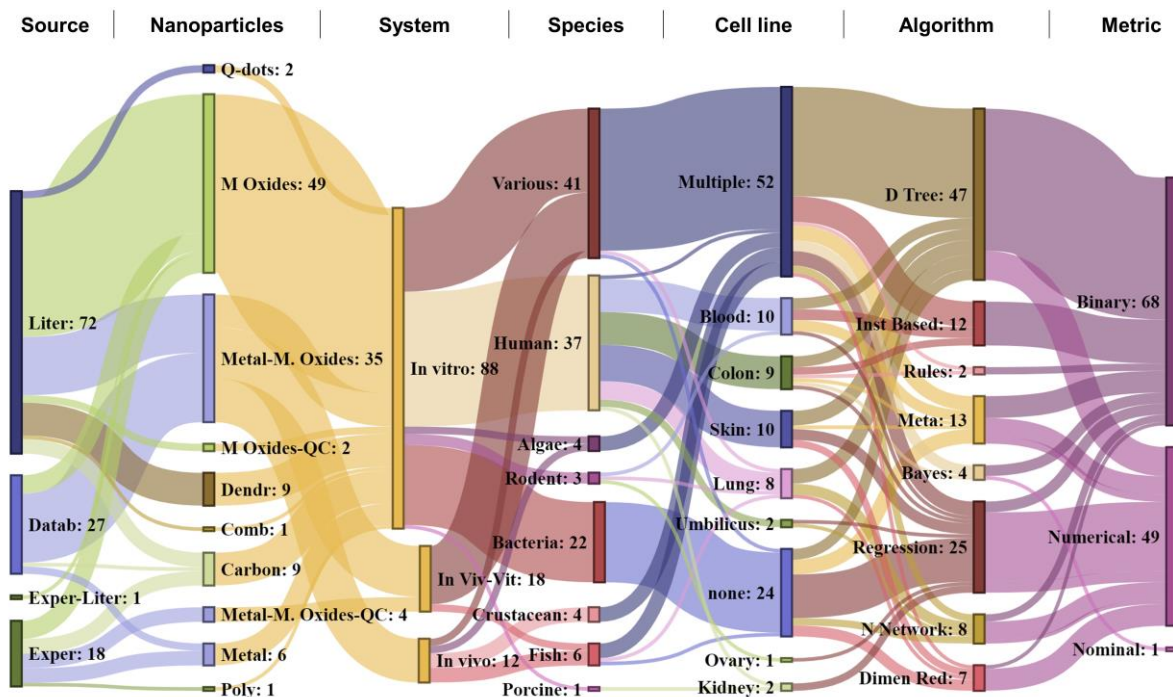
495 **Figure 9. Case flow by attribute, featuring membrane integrity as outcome.** The width of  
 496 the flows is proportional to the number of cases. The flow demonstrates what cell lines of which  
 497 species were exposed to which particles, in *in vivo* or *in vitro* experiments, resulting in affecting  
 498 membrane integrity. It also shows what models were used for predicting membrane integrity,  
 499 codified as either a binary, numerical or nominal outcome. *Liter*: literature, *Exper*: experimental.

500 Cases predicting *membrane integrity* are shown in **Figure 9**. Models to predict *membrane*  
 501 *integrity* were applied for metal oxides (12 cases) and carbon based NP (2 cases) for *in vitro*  
 502 and *in vivo* systems, respectively. Five cases contained new experimental data and nine cases  
 503 contained literature datasets. Twelve out of 14 assays involve human and rodent lung cells and  
 504 two involve liver cells. Gernand and Casman (2014) presented a regression-*tree* QSAR on a  
 505 meta-analysis of rodent pulmonary studies of uncoated carbon nanotubes exposure. Individual  
 506 endpoints were measured in bronchoalveolar lavage fluid of the mice or rats covering  
 507 polymorphonuclear neutrophils, macrophages, lactate dehydrogenase (LDH) and total protein.  
 508 Those endpoints associated with immune response, membrane damage and cell death were  
 509 expressed in numeric form (fold of control measurements). Le, *et al.* (2016) studied ZnO NPs  
 510 exposure using biological assays measuring cell damage or stress of human umbilical cells or

511 human liver. *Regression* and *neural network* models were developed to link NP features to cell  
512 viability, membrane integrity and oxidative stress. Membrane integrity was determined by LDH  
513 assay and the endpoint was predicted in numeric by plasma-membrane leakage via PI uptake  
514 against population not exposed to NPs. Liu, *et al.* (2011) SAR model predicted the *membrane*  
515 *integrity* in a binary form using a decision boundary to divide the input parameter space into  
516 toxic and nontoxic regions.

517 Toropova, *et al.* (2015b) using data from (Patel, *et al.*, 2014) created a QSAR model with  
518 optimal descriptors (quasi-SMILES) for the prediction of membrane damage by propidium  
519 iodide uptake by metal-oxides. Sayes and Ivanov (2010) attempted to identify a set of properties  
520 of TiO<sub>2</sub> and ZnO to describe and estimate the damage (via LDH release) of human lung cells  
521 implementing *dimensionality reduction* and *regression* QSARs. LDH release was categorized  
522 into four different nominal impact potentials e.g., normal or leaky (2 cases with nominal  
523 outcomes as shown in **Figure 9**). Toropova and Toropov (2013) used experimental data from  
524 (Sayes and Ivanov, 2010) with optimal descriptors for the prediction of membrane damage  
525 (units L<sup>-1</sup>) for various TiO<sub>2</sub> by means of quasi-QSAR. Papa, *et al.* (2015) developed linear  
526 (*regression*) and non-linear (*decision tree*, *neural network*) QSAR modelling of the cytotoxicity  
527 of TiO<sub>2</sub> and ZnO using the original experimental descriptors from (Sayes and Ivanov, 2010).  
528 They distinguished toxic (leaking or disrupted membrane) instances using an LDH cut-off value  
529 of 1.09.

530 In conclusion, several linear and non-linear models have been applied for the prediction of  
531 *membrane integrity* in different metrics (binary, nominal, numerical), mostly for metal oxides  
532 (mostly ZnO and TiO<sub>2</sub>) in human lung cells. *Decision tree* models were used for *in vivo* rodent  
533 pulmonary data and carbon based NPs.



534

535 **Figure 10. Case flow by attribute, featuring cell viability as outcome.** The width of the flows is  
 536 proportional to the number of cases. The flow demonstrates what cell lines of which species are  
 537 exposed to which particles, in *in vivo* or *in vitro* experiments, resulting in affecting cell viability. It also  
 538 shows what models were used for predicting cell viability, codified as either a binary, numerical or  
 539 nominal outcome. *Liter*: literature, *Exper*: experimental, *Datab*: database, *Q-dots*: quantum-dots, *Dendr*:  
 540 dendrimers, *Poly*: polymers, *comb 1* dataset containing carbon-based, metal, metal oxides, polymeric and  
 541 dendrimers NPs, *in viv-vit*: dataset containing *in vivo* and *in vitro* data.

542 The majority of the studies modeling *viability* extracted information from literature (72  
 543 cases). 27 cases were created using data from databases such as S2NANO and OCHEM. The  
 544 most common NPs used in the datasets were metal oxides (49 cases) and combination of  
 545 metals/metal oxides (35 cases). *In vitro* systems usually considered various species and multiple  
 546 human cell lines (**Table 4**). For *viability*, a plethora of eco-toxicological endpoints using algae,  
 547 bacteria, fish and crustacean were used compared to other outcomes. The majority of non-linear

548 models predicted *viability* in a binary form (68 cases) the rest in numeric (49 cases) (**Figure**  
549 **10**).

550

551 **Table 4. Species (various in Figure 10) and cell lines (multiple in Figure 10) examples.**

<b>Various (species)</b>	Human, mice, rat, organisms, bacteria, mouse, hamster, fish, dog, monkey, murine, rat, pig, canine, cow, among others.
<b>Multiple (cell lines or species details)</b>	lung, liver, kidney, intestine, bone marrow, areolar, prostate, heart, ovary, aorta, foreskin, gland, stomach, testis, urinary bladder, lymph node, lymphocyte intestinal, cardiovascular, alveolar macrophage, blood, glioma, bone, skin, brain, embryo, fibroblast, breast, colon, neutrophils, somatic cell hybrid, cervix, umbilical vein, dendritic cells, Staphylococcus aureus, Escherichia coli, zebrafish, Daphnia magna, Danio rerio, Pseudokirchneriella subcapitata, among others.
<b>NPs (Combina- tions)</b>	Comb1: dataset containing carbon-based, metal, metal oxides, polymeric and dendrimers NPs.

552 Regarding data from databases, Trinh, *et al.* (2018b) performed data curation and meta-analysis  
553 for metals NPs to predict *viability* in a binary form (cut-off value of 50%). They implemented  
554 *decision tree* (Random Forest, RF) and *instance based* (SVM) algorithms using several *in vitro*  
555 cell lines from different species, p-chem properties and experimental conditions. Trinh, *et al.*  
556 (2018) predicted the impact of carbon-based NPs on *viability* of human lung cell lines using  
557 SMILES-based QSAR and RF. Metal oxides from the S2NANO database and several cell lines  
558 were tested implementing linear and non-linear modelling to predict *viability* either in binary  
559 (Choi, *et al.*, 2018) or numeric form (Choi, *et al.*, 2019). The above studies provided the curated  
560 datasets as supplementary material (see supplementary material for studies that included the

561 datasets used). Kovalishyn, *et al.* (2018) gathered data on NPs toxicity in different organisms  
562 and cell lines and uploaded them on OCHEM. They used *decision tree*, *instance based* and  
563 *neural network* algorithms to predict *viability* in a binary form. Puzyn, *et al.* (2011) used new  
564 data generated experimentally and data from (Hu, *et al.*, 2009) to predict numeric bacteria  
565 *viability* ( $\log_1/EC_{50}$ ) of metal oxides. They developed a QSAR model using multiple *regression*  
566 combined with a genetic algorithm (Puzyn, *et al.*, 2011). The genetic algorithm selected the  
567 optimal descriptors from the ones already calculated by the regression model.

568 The majority of the models were based on *in vitro* assays (88 cases) as shown in **Figure 10**, and  
569 a portion (18) were based on combination of *in vivo/in vitro* experiments. Twelve cases focused  
570 on *in vivo* systems predicting binary *viability* after exposure on metals/metal oxides  
571 (Kovalishyn, *et al.*, 2018, Chen, *et al.*, 2016). Chen, *et al.* (2016) studied hazard categorization  
572 for regulatory purposes using SARs based on information gathered from OCHEM. They  
573 retrieved multi-source eco-toxicity data and applied four *decision tree* algorithms across  
574 (various) species and species-specific (fish, bacteria) models. They predicted binary *viability*  
575 defined on an arbitrary threshold (active and inactive from  $LC_{50}$ ,  $EC_{50}$  and MIC data).  
576 Numerous studies focused in *viability* used data derived from assays of different cell lines from  
577 various species (human, fish, algae, rodent, bacteria, etc., see **Table 4**).

578 Toropova, *et al.* (2015) predicted *viability* for  $SiO_2$  using data from human and porcine renal  
579 tubular kidney cells applying quasi-QSAR model based on eclectic data and SMILES. Hamster  
580 ovary cell line data were used for the numerical prediction of  $TiO_2$  NPs  $\log_1/EC_{50}$  *viability*  
581 using multiple linear *regression* analysis in (Mikolajczyk, *et al.*, 2018). Four *bayesian networks*  
582 cases were developed to predict viability of quantum dots (Bilal, *et al.*, 2019), metal oxides  
583 (Furxhi, *et al.*, 2019a) and dendrimers (Jones, *et al.*, 2015). While *bayesian networks* offer many  
584 advantages such as, visual representation of the model, correlation among inputs, user  
585 friendliness and good performance with missing values they seem to have been poorly exploited

586 in the field (Murphy, *et al.*, 2016, Marvin, *et al.*, 2017, Furxhi, *et al.*, 2019b). (Bilal, *et al.*, 2019)  
587 predicted numerical *viability* of Cd-containing quantum dots as percentage and IC<sub>50</sub> (expressed  
588 into log<sub>10</sub>) using *Bayesian Networks*, including web-based model versions with outcomes  
589 discretized in bands. Jones, *et al.* (2015) tested the ability of a variety of algorithms (*Bayesian*  
590 *Networks, meta, regression, instance based, rules*) to predict binary *viability* of human colon  
591 cells exposed to poly(amido amine) (PAMAM) dendrimers.

## 592 **5 Discussion**

593 In this paper, we provided an overview of nanotoxicology cases that implement machine  
594 learning methods to predict human health and ecotoxicological hazard endpoints, highlighting,  
595 for the top five most predicted outcomes the biological level of organization. We did not  
596 compare machine learning algorithms and the actual results of the models. Different approaches  
597 cannot be easily compared due to the diversity of input choices and datasets, even for the same  
598 outcome. However, several methodologies have been proposed to rank and compare classifiers  
599 according to their predictive performance (Furxhi, *et al.*, 2019a, Tamvakis, *et al.*, 2018, Tsiliki,  
600 *et al.*, 2015).

601 The studies presented here were not reviewed regarding their compliance to the fifth OECD  
602 principle, that is, having included a mechanistic interpretation; the goal of the manuscript was  
603 not to critically assess available tools but rather to provide a synthetic overview of the machine  
604 learning tools in nanotoxicology based on the biological organization level.

### 605 **5.1 Practices of current studies**

#### 606 **5.1.1 Data sources in the studies**

607 The majority of the reviewed studies extracted data from peer-reviewed literature sources  
608 as demonstrated in section 4.1. Available computational approaches were not build as much on  
609 recognized databases such as S2NANO, NIOSH, OCHEM, NBI etc., while one study used data

610 from eNanoMapper (Helma, *et al.*, 2017). This can be attributed to the fact that most of those  
611 databases are, for the moment, not nano-specific e.g., the OCHEM database contains nano and  
612 non-nano experimental data. ENanoMapper is an ongoing project, integrating research data  
613 from various relevant projects and literature, expected to deliver results in the near future. Most  
614 endpoints derived from databases were used for modeling ecotoxicity. This indicates that there  
615 are more data publicly available suitable for ecotoxicity endpoints than human health hazard  
616 endpoints. Over 200 *in vivo* assessments for NP toxicity in embryonic zebrafish model can be  
617 found in the NBI. Almost all database derived data regard metal and metal oxide NPs.

#### 618 5.1.2 *Nanoparticle and study design information*

619 There has been a trend of developing toxicity models for metal oxides and metals or  
620 combined metals/metal oxides NPs as demonstrated in section 4.1, due to the growing  
621 popularity of using metal and metal oxide NP in commercial products (Vance, *et al.*, 2015).  
622 Still, there are no studies reporting cytotoxicity prediction for quite a few NP types such as  
623 micelles, liposomes, dendrimers, mixtures and polymeric NPs. Carbon-based, polymeric or  
624 dendrimer NP eco-toxicity modelling is also missing.

625 Roughly half of the approaches reviewed included nanospecific descriptors in the final model,  
626 while the other half included calculated theoretical descriptors as input variables, either used as  
627 such, or in combination with p-chem characteristics. The most common p-chem attributes  
628 include size either as primary or aggregated size, zeta potential and surface area. *In vitro*  
629 experimental conditions such as cell type, origin or toxicological assay were less commonly  
630 included. Size, shape, zeta potential, surface area and composition are among the most  
631 commonly used, or recommended to be used, nano-features for a toxicological evaluation  
632 though a broad scientific consensus on the set of required features is still missing (Oksel, *et al.*,  
633 2015b). Defining the significant drivers of toxicity among the various NP p-chem is challenging  
634 for a number of reasons as summarized in (Ribeiro, *et al.*, 2017). Associating p-chem properties

635 to toxicity is demanding as (eco) toxicity can be affected by changes in a property in a non-  
636 trivial manner. It is worth noting that the new, relevant to nanoforms, revisions of the Annexes  
637 of the European Union's chemical legislation demand that, quoting Clausen and Hansen (2018),  
638 “*Thou shalt not use molecular structural similarities alone as a justification for grouping*  
639 *different nanoforms*”. This decree signifies the importance of incorporating nano-specific  
640 features in a model.

641 The majority of the studies created *in silico* tools from *in vitro* toxicological data as  
642 demonstrated in section 4.2. One of the general limitations of *in vitro* test systems is that they  
643 are restricted to one or a few different cell types and, thus, cannot represent the biological  
644 responses in the whole organism. Furthermore, these systems are usually derived from cancer  
645 cell lines which can result in different outcomes when compared to *in vivo* tests. To tackle this  
646 issue, cell type and cell origin can be accounted for as input variables in the model (Choi, *et al.*,  
647 2018, Furxhi, *et al.*, 2019a). Evidently, the number of *in vivo* modelling was low; 10 out of 86  
648 studies were performed with an *in vivo* system (rodent, fish, crustacean or combination of  
649 organisms). Those tendencies reflect ethical and economic considerations, channeling research  
650 resources towards the development of toxicity test alternatives, such as *in vitro*.

651 *Aggregated* outcomes prediction is mostly found in cases of various species (human and  
652 ecotoxicological) and multiple cell lines, as demonstrated in section 4.2. *Apoptosis* outcome  
653 models used human cell lines of liver, aorta, blood and muscle. *Cellular uptake* is predicted  
654 mostly for human pancreatic cell lines while membrane integrity is commonly predicted for  
655 rodent lung cell lines. For the three latter outcomes, there has been no tool developed for  
656 ecotoxicological prediction. *Viability*, the most abundantly predicted outcome using machine  
657 learning is predicted for various ecotoxicological species, human and various other species, in  
658 multiple cell lines such as lung, colon, blood and skin. A few models used *in vitro* experimental  
659 data, such as cell type, cell line or cell origin, as inputs. Consequently, most models applied for

660 *in vitro* studies will fall short to capture the impact on the predicted toxicity of the experimental  
661 conditions variations, corresponding to differences in biological organization levels. It is  
662 notable that no machine learning tool was applied to predict neurotoxicological outcomes.

663 Single exposures are typically used in *in vitro* studies which usually last from a few minutes up  
664 to a few days depending on the endpoint tested. Therefore, chronic exposure cannot be tested  
665 sufficiently. Defining a suitable dose range is a challenge for both *in vitro* and *in vivo* tests as  
666 often unrealistically high doses are chosen in order to observe an effect. As demonstrated in  
667 section 4.1, exposure dose and duration have not been frequently used as input variables and  
668 their effect on the predicted outcome has not been appropriately modelled. When dose is  
669 included, it is ranked as a significant feature for the prediction of the outcome (Choi, *et al.*,  
670 2018, Furxhi, *et al.*, 2019b).

### 671 5.1.3 *Studies Endpoints*

672 A well-defined endpoint is essential for clarity regarding what is being predicted by a  
673 model. A range of specific considerations for evaluating an endpoint include the scientific  
674 purpose, the quality of the assays and experimental protocol(s); the units of measurement, the  
675 underlying data and the regulatory relevance of the endpoint (Puzyn, *et al.*, 2018). QSARs were  
676 developed mostly for cytotoxicity endpoints which are not endpoints included in REACH.  
677 REACH toxicological endpoints such as on acute toxicity, repeated dose toxicity, sensitization,  
678 carcinogenicity or reproductive toxicity are not covered by models developed so far. The most  
679 common endpoints evaluated in literature were measured as cellular viability, membrane  
680 damage, cellular uptake and apoptosis, as shown in section 4.1. All relevant models for cellular  
681 uptake used the same original dataset which describes the cellular uptake of cross-linked iron  
682 oxide NPs by pancreatic human cancer cells. Generic aggregated endpoints were commonly  
683 used, an example of the trade-off between data availability and data quality. One third of the  
684 approaches reviewed corresponded to combinations of endpoints.

#### 685 5.1.4 Model implementation

686 *Trees, neural network and regression* algorithms are abundant compared to *rules, bayes,*  
687 or *meta* algorithms as demonstrated in section 4.2. Most of the modelling, for all categories of  
688 NPs, has focused on the traditional implementation of QSAR or SAR. There are two alternative  
689 approaches namely quasi-QSARs and perturbation models that compared to classical models,  
690 handle and exploit available data differently. Those promising approaches focus on extracting  
691 more information from datasets than traditional approaches do and meet the latest regulation  
692 requirements as they incorporate several experimental features.

693 Experimental conditions affect the observed outcomes, hence it is essential to include this  
694 dependent information in a QSAR model. In quasi-QSARs the endpoint is associated to NPs  
695 through a mathematical function of available eclectic data; those data include biochemical  
696 parameters (synthesis), exposure conditions (dose and duration of exposure, bio targets such as  
697 cells, species, organisms) and p-chem properties that affect the outcome (Toropova, *et al.*,  
698 2015b, Choi, *et al.*, 2019). In contrast, traditional models are mathematical functions of  
699 molecular structures. In quasi-QSAR the eclectic data are represented in the quasi-Simplified  
700 Molecular Input-Line Entry System (SMILES) which comprises character-based strings  
701 representations of a series of simple syntactic sequences (Trinh, *et al.*, 2018). In the case of  
702 continuous data, the data can be normalized and discretized into subintervals. As the number of  
703 codes in the quasi-model decreases so does the sensitivity of quasi-QSAR (Toropova, *et al.*,  
704 2015). An improved quasi-SMILES code assignment method was proposed to tackle the issue  
705 using hierarchical cluster analysis (Trinh, *et al.*, 2018). Similarity between numerical  
706 descriptors is used to group the codes to a number of groups before the eclectic data are  
707 translated into optimal nano-descriptors (the sum of weights of the quasi-SMILES). An  
708 alternative to calculate the optimal descriptors is using Monte Carlo from arbitrary eclectic  
709 information. The approach is attractive as descriptors can be easily modified for novel

710 experimental data. The quasi-SMILES provide a suitable framework for developing predictive  
711 models for NPs since all available eclectic data that impact toxicity can be integrated and the  
712 impact of descriptors on toxicity can be evidently seen (Choi, *et al.*, 2019). Using eclectic data  
713 increases the number of available datasets which is a fair advantage of quasi-SMILES in the  
714 case of nanotoxicology lack of data.

715 Until now, QSARs and quasi-QSARs have attempted the prediction of one outcome usually  
716 against one biological entity/system (e.g. cell line, fish, bio-indicator etc.) based on classical  
717 approaches and small datasets. However, there is a need for models that predict the toxicity of  
718 NPs in different organisms/bio-indicators and diverse experimental conditions. A promising  
719 approach based on perturbation theory has been proposed recently (Kleandrova, *et al.*, 2014,  
720 Kleandrova, *et al.*, 2017) which in conjunction with quasi-models can i) combine multiple nano-  
721 descriptors (p-chem properties, key biological factors, interspecies, experimental conditions)  
722 using the moving average approach and ii) simultaneously predict several multiple toxicity  
723 (cytotoxicity and ecotoxicity) endpoints. Considering the large number of new NPs and the  
724 safety obligations from regulatory agencies requiring toxicity data in multiple species or  
725 toxicity endpoints, perturbation models are able to handle interspecies prediction of multiple  
726 toxicity endpoints. This significantly limits resources demands, including time needed for  
727 evaluating the toxicity of new NPs (bridging data gaps) and may speed up regulatory decisions  
728 (Luan, *et al.*, 2014, Basant and Gupta, 2017). Interspecies modeling reduces testing on higher  
729 level organisms and contributes to mechanism of action interpretation (De, *et al.*, 2018). In  
730 classic QSARs and quasi-QSARs each case constitutes one single prediction in the data set and  
731 the intercept of the equation is often considered as the value of reference. Any case, though,  
732 can be used as reference and neglecting this affects model reliability (Kleandrova, *et al.*, 2014b).  
733 Perturbation models use random pairs of features where each feature is used many times as  
734 input (reference) and as output (prediction) and is predicted many times from the others.

735 Contrary to classical models this leads to more realistic predictions and at the same time  
736 increases the number of data cases dramatically (Kleandrova, *et al.*, 2014, Kleandrova, *et al.*,  
737 2017). The moving average approach uses perturbations of traditional features (molecular  
738 information) around same features averages in different experimental conditions in order to  
739 calculate a new set of toxicological encoded descriptors (González-Durruthy, *et al.*, 2017). In  
740 addition, by using relative values instead of absolute ones this methodology is sensitive to the  
741 finer modifications of descriptors, thus offering refined information compared to traditional  
742 data handling. (González-Durruthy, *et al.*, 2017). While perturbation models mentioned in the  
743 literature has been mostly linear, as the interaction of NPs with biological systems is complex,  
744 novel perturbation models using non-linear approaches (*neural networks*) has been also  
745 developed (Concu, *et al.*, 2017).

746 Simultaneous prediction of multiple outcomes has been achieved by another non-linear  
747 algorithm, *bayesian networks* (Furxhi, *et al.*, 2019b, Furxhi, *et al.*, 2018). The authors used  
748 feature correlation results among p-chem properties, experimental exposure conditions and *in*  
749 *vitro* characteristics in order to manually craft a Bayesian structure, similarly to (Bilal, *et al.*,  
750 2019). They predicted nine different biological effects on a molecular level using outcomes of  
751 transcriptomics studies such as cell cycle and proliferation responses or cell death and apoptosis  
752 responses. That was the first non-perturbation model that simultaneously predicted outcomes  
753 without using aggregated nodes that cluster the toxicological effects into one class. Clustering  
754 several effects in one outcome, the norm for *Bayesian Networks* application in nanotoxicology,  
755 disregards the differentiation of attribute importance in predicting different toxicological  
756 endpoints (Murphy, *et al.*, 2016, Marvin, *et al.*, 2017).

## 757 5.2 Challenges and Perspectives

### 758 5.2.1 Qualities and integration

759 Predicting (eco) toxicological effects of NPs by means of *in silico* tools requires access  
760 to high quality (meta) data. Data gathered or generated can be reused from different  
761 stakeholders; however, only 27% of studies provided accessibility of data in supplementary  
762 spreadsheets. Cell culture conditions and methodology, NP preparation and all elements and  
763 stages of experimental design and laboratory conditions should be clearly described either when  
764 set to be used by QSAR or by any computational tool, to ensure compliance to the second  
765 OECD principle, enabling interpretability of data and comparability with other studies (Puzyn,  
766 *et al.*, 2018). Still, the question of experimental quality guidelines, standard procedures,  
767 sufficient characterization, dosimetry issues and the “reproducibility crisis” remain in the field  
768 (Donaldson, *et al.*, 2013, Poland, *et al.*, 2014, França and Monserrat, 2018). (Oksel, *et al.*, 2017,  
769 Oksel, *et al.*, 2015b) provided a critical review of the availability of NP characterization and  
770 toxicity data meeting the requirements for *in silico* analysis. The data curation topic, one of the  
771 major issues highlighted in recent review papers of nanotoxicity prediction, has been studied  
772 by a number of collaborative consortia. A series of studies focused on aspects of data curation,  
773 such as the workflow, data completeness, quality and the role of data curation in  
774 nanoinformatics (Hendren, *et al.*, 2015, Powers, *et al.*, 2015). This review does not critically  
775 assess data curation issues, techniques or methods, but stresses their importance. Those issues  
776 are addressed elsewhere, especially in the Nanomaterial Data Curation Initiative (NDCI)  
777 (Basei, *et al.*, 2019, Trinh, *et al.*, 2018b, Hendren, 2015, Karcher, *et al.*, 2018, Quik, *et al.*,  
778 2018).

779 ISA-TAB-nano (and upgrades to ISA-JSON) standard formats were developed aiming at  
780 creating a global authoritative database for easily accessible and transferable data from different  
781 databases, laboratories and researchers (Thomas, *et al.*, 2013). However, no tools to parse the

782 files and to enforce the specification are available, as demonstrated by the introduction of “ISA-  
783 Tab-logic” templates (Haase, 2018). Marchese Robinson, *et al.* (2015) provided a generic ISA-  
784 TAB-Nano that serve as a starting point for the construction of nanotoxicology datasets.  
785 However, the authors note that guidance regarding the different kinds of (meta)data is required,  
786 due to the necessity of having p-chem and toxicological information in one dataset, in order to  
787 facilitate the predictive nanotoxicology and to enable safe-by-design approaches. Thus, it would  
788 be wise, for any new experimental data generated to use a nano-format to enable future  
789 integration to either datasets or databases. An integration and a reuse of data will also  
790 necessitate common terminologies; several tools have already been developed using ontologies  
791 to integrate different data sources. Haase (2018) provided an overview of generic ontology  
792 tools. Córdoba and Zambon (2017) proposed a novel ontological category of nanoindividuals,  
793 as a recommendation for the stepwise procedure for chemical categories proposed by OECD.  
794 The databases will finally integrate into a single database with highly curated (meta) data  
795 available for computational toxicology. This course will eventually lead to the acceptance of *in*  
796 *silico* tools in regulatory bodies in compliance with the 3Rs movement. Datasets and/or  
797 databases integration will also generate new hypotheses and knowledge and will benefit  
798 multiple stakeholders (Karcher, *et al.*, 2018). In the paper *ibid*, the authors highlight the  
799 importance of data integration in nanotechnology and provide recommendations for advancing  
800 integration.

801 Haase (2018) provided a summary of available nano-specific databases. In parallel, Basei, *et*  
802 *al.* (2019) provided 16 nano-specific databases while critically addressing their accessibility to  
803 the public and data containment. Karcher, *et al.* (2018) also commented on accessibility,  
804 suggesting practices to enhance the process of integrating data sources, taking into account

805 ontologies. GRACIOUS<sup>14</sup>, an ongoing project, will integrate available databases into a singular  
806 database<sup>15</sup> addressing data curation to develop a scientific framework for grouping, read-across  
807 and classification of NPs. Similar efforts for curating and integrating existing data are being  
808 conducted in international projects such as NanoFASE<sup>16</sup>, Nano-Commons<sup>17</sup> and ACENano<sup>18</sup>.

809 Another database, the S2NANO<sup>19</sup>, although including various experimental results related to  
810 NPs obtained from different sources achieved little visibility in recent review studies (Lamon,  
811 *et al.*, 2019, Basei, *et al.*, 2019, Saini and Srivastava, 2018). S2NANO addresses issues of data  
812 quality and completeness using a p-chem score screening system and a data gap filling method  
813 based on manufacturer's specifications and/or estimations (Trinh, *et al.*, 2018b, Ha, *et al.*,  
814 2018).

815 Similar to S2NANO, platforms like eNanoMapper are being created as means for collection  
816 and curation of data to establish completeness and quality (Basei, *et al.*, 2019). The Cancer  
817 Nanotechnology Laboratory data portal (caNanoLab)<sup>20</sup> is a collaborative effort between three  
818 NCI interdisciplinary research centers, to integrate global research data sources. caNanoLab  
819 serves as a data repository that allows researchers to submit and retrieve information on well-  
820 characterized NPs including compositions; p-chem, *in vitro* and *in vivo* characteristics,  
821 associated publications and assay protocols (Grodzinski, *et al.*, 2019).

### 822 5.2.2 Emerging infrastructures

823 Concerning the sparsity and heterogeneity of available data, the EU has funded projects to  
824 address data quality and accessibility. Concurrently, the integration of computational platforms

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<sup>14</sup> <https://www.h2020gracious.eu/> (Webpage accessed autumn 2019).

<sup>15</sup> (<https://apps.ideaconsult.net/gracious/ui>) (Webpage accessed autumn 2019).

<sup>16</sup> <http://www.nanofase.eu/> (Webpage accessed autumn 2019).

<sup>17</sup> <https://www.nanocommons.eu/> (Webpage accessed autumn 2019).

<sup>18</sup> <http://www.acenano-project.eu/> (Webpage accessed autumn 2019).

<sup>19</sup> (<http://portal.s2nano.org/>) (Webpage accessed autumn 2019).

<sup>20</sup> <https://cananolab.nci.nih.gov/> (Webpage accessed autumn 2019).

825 will allow different stakeholders to explore data by means of *in silico* tools. H2020  
826 NanoCommons<sup>21</sup>, a new project, addresses data accessibility (data on toxicity, characterization,  
827 protocols etc.). The project applies quality assurance criteria while underpinning ontologies to  
828 create an *in silico* framework and infrastructure for NP safety assessment. NanoInformaTIX<sup>22</sup>  
829 develops a platform for risk management of NP based on p-chem and toxicological data already  
830 generated, as well as data currently produced in ongoing projects. The data will be stored in a  
831 database operated by the project. NanoSolveIT<sup>23</sup> will collect publicly available data from  
832 different sources, curate, harmonize, FAIRify (assure Findability, Accessibility,  
833 Interoperability, and Reusability) and integrate existing and emerging data on characterization,  
834 release, exposure, biological and toxicological effects on human health and the environment.  
835 In addition, targeted datasets will be delivered to gap-fill incomplete datasets and models after  
836 collation and procurement of all data available via consortium partners.

837 Nano-specific databases are still in research stage. Data are generated experimentally following  
838 different procedures. In order to build reliable datasets standard protocols should be followed,  
839 datasets should be adequately large and values should be suitable for computational use  
840 (Villaverde, *et al.*, 2018). There is a significant amount of nano-relevant datasets unstructured  
841 and scattered. There are ongoing efforts to fuse data from different sources, facing difficulties  
842 due to lack of standardized access and broad agreement on codifications of NPs and related  
843 data. Common language is being developed, but the computational nanotoxicology community  
844 is still developing consensus on conceptualization and communication of the field elements and  
845 their associations.

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<sup>21</sup> <https://www.nanocommons.eu/> (Webpage accessed autumn 2019).

<sup>22</sup> <http://www.nanoinformatix.eu/> (Webpage accessed autumn 2019).

<sup>23</sup> <https://nanosolveit.eu/> (Webpage accessed autumn 2019).

## 846 **6 Conclusion**

847 This literature review in nanotoxicology field predicting human health and eco-toxicological  
848 endpoints identified several machine learning models that provide prediction to numerous  
849 nanotoxicological outcomes and supports the notion that data availability and curation are still  
850 the main issues faced by the computational toxicology community. The main conclusion of our  
851 analysis is that most studies perform modelling for few NPs, extracting data mainly from the  
852 literature and test in various *in vitro* systems and multiple cell lines. Several novel  
853 computational approaches such as perturbation models or quasi-QSARs can help to build  
854 biologically more accurate models since they capture the impact on the outcome of exposure  
855 conditions and other experimental parameters. Perturbation models also provide a solution for  
856 small datasets, which is a major issue in *in silico* approaches. Databases facilitation and  
857 multisource data extraction is still under development but set recently as a priority in research  
858 community, so highly curated data are expected to be shortly available blooming the area of  
859 computational nanotoxicology. Ongoing efforts and further research will advance as  
860 computational tools are developed and implemented and might shade light in the key properties  
861 that ultimately affect NPs toxicity, offering safe-by-design criteria for a promising  
862 nanotechnological era.

## 863 **7 Acknowledgements**

864 Not applicable

## 865 **8 Declaration of interest statement**

866 The authors report no conflict of interest.

## 867 **Funding**

868 This work was supported by the European Union's Horizon 2020 research and innovation in  
869 SMEs program under Grant Number 720851, project PROTECT. Craig A. Poland was  
870 supported by the Colt Foundation (project CF/01/17).

## 871 **9 References**

872 A. McWilliams. (2017). Markets of magnitude Singapore, Malaysia, Thailand, and Indonesia take the  
873 lead in Southeast Asian ceramic technology research, development, and cross-border collaboration.  
874 American Ceramic Society Bulletin. United States of America: The American Ceramic Society, 1–56.

875 A. Giusti, R. Atluri, R. Tsekovska, A. Gajewicz, M. D. Apostolova, C. L. Battistelli, E. A. J. Bleeker,  
876 C. Bossa, J. Bouillard, M. Dusinska, P. Gómez-Fernández, R. Grafström, M. Gromelski, Y.  
877 Handzhiyski, N. R. Jacobsen, P. Jantunen, K. A. Jensen, A. Mech, J. M. Navas, P. Nymark, A. G.  
878 Oomen, T. Puzyn, K. Rasmussen, C. Riebeling, I. Rodriguez-Llopis, S. Sabella, J. R. Sintes, B. Suarez-  
879 Merino, S. Tanasescu, H. Wallin and A. Haase. 2019. Nanomaterial grouping: Existing approaches and  
880 future recommendations. *NanoImpact* 16:100182.

881 R. Chen, J. Qiao, R. Bai, Y. Zhao and C. Chen. 2018. Intelligent testing strategy and analytical  
882 techniques for the safety assessment of nanomaterials. *Analytical and Bioanalytical Chemistry*  
883 410:6051-6066.

884 T. Burgdorf, A. H. Piersma, R. Landsiedel, R. Clewell, N. Kleinstreuer, M. Oelgeschläger, B. Desprez,  
885 A. Kienhuis, P. Bos, R. de Vries, L. de Wit, T. Seidle, J. Scheel, G. Schönfelder, J. van Benthem, A. M.  
886 Vinggaard, C. Eskes and J. Ezendam. 2019. Workshop on the validation and regulatory acceptance of  
887 innovative 3R approaches in regulatory toxicology – Evolution versus revolution. *Toxicology in Vitro*  
888 59:1-11.

889 ECHA. (2017). Non-animal approaches - Current status of regulatory applicability under the REACH,  
890 CLP and Biocidal Products regulations. 163.

891 ECHA. (2017b). The use of alternatives to testing on animals for the REACH Regulation. 103.

892 K. Herrmann and K. Jayne. (2019). *Animal Experimentation: Working Towards a Paradigm Change*.  
893 Brill.

894 C. Schwarz-Plaschg, A. Kallhoff and I. Eisenberger. 2017. Making Nanomaterials Safer by Design?  
895 *NanoEthics* 11:277-281.

896 A. Kraegeloh, B. Suarez-Merino, T. Sluijters and C. Micheletti. 2018. Implementation of Safe-by-  
897 Design for Nanomaterial Development and Safe Innovation: Why We Need a Comprehensive  
898 Approach. *Nanomaterials* 8:239.

899 T. Puzyn, N. Jeliaskova, H. Sarimveis, R. L. Marchese Robinson, V. Lobaskin, R. Rallo, A.-N. Richarz,  
900 A. Gajewicz, M. G. Papadopoulos, J. Hastings, M. T. D. Cronin, E. Benfenati and A. Fernández. 2018.  
901 Perspectives from the NanoSafety Modelling Cluster on the validation criteria for (Q)SAR models used  
902 in nanotechnology. *Food and Chemical Toxicology* 112:478-494.

903 A. Haase, & Klaessig, F., (2018). EU US Roadmap Nanoinformatics 2030. EU Nanosafety Cluster.

- 904 A. K. Worth A., Asturiol B. D., Bessems J., Gerloff K. B., Graepel R., Joossens E., Lamon L., Palosaari  
905 T., Richarz A., (2017). Evaluation of the availability and applicability of computational approaches in  
906 the safety assessment of nanomaterials: Final report of the Nanocomput project.
- 907 OECD. (2009). Guidance document on the validation of (quantitative)structure-activity relationships  
908 [(Q)SAR] models., 1-154.
- 909 M. Li, P. Zou, K. Tyner and S. Lee. 2017. Physiologically Based Pharmacokinetic (PBPK) Modeling of  
910 Pharmaceutical Nanoparticles. *The AAPS Journal* 19:26-42.
- 911 D. Yuan, H. He, Y. Wu, J. Fan and Y. Cao. 2019. Physiologically Based Pharmacokinetic Modeling of  
912 Nanoparticles. *Journal of Pharmaceutical Sciences* 108:58-72.
- 913 L. Lamon, D. Asturiol, A. Richarz, E. Joossens, R. Graepel, K. Aschberger and A. Worth. 2018.  
914 Grouping of nanomaterials to read-across hazard endpoints: from data collection to assessment of the  
915 grouping hypothesis by application of chemoinformatic techniques. *Particle and Fibre Toxicology*  
916 15:37.
- 917 L. Lamon, K. Aschberger, D. Asturiol, A. Richarz and A. Worth. 2018b. Grouping of nanomaterials to  
918 read-across hazard endpoints: a review. *Nanotoxicology*:1-19.
- 919 I. Furxhi, F. Murphy, M. Mullins, A. Arvanitis and C. A. Poland. 2020b. Practices and Trends of  
920 Machine Learning Application in Nanotoxicology. *Nanomaterials* 10:116.
- 921 L. Lamon, D. Asturiol, A. Vilchez, R. Ruperez-Illescas, J. Cabellos, A. Richarz and A. Worth. 2019.  
922 Computational models for the assessment of manufactured nanomaterials: Development of model  
923 reporting standards and mapping of the model landscape. *Computational Toxicology* 9:143-151.
- 924 R. Weissleder, K. Kelly, E. Y. Sun, T. Shtatland and L. Josephson. 2005. Cell-specific targeting of  
925 nanoparticles by multivalent attachment of small molecules. *Nature biotechnology* 23:1418-1423.
- 926 T. Puzyn, B. Rasulev, A. Gajewicz, X. Hu, T. P. Dasari, A. Michalkova, H.-M. Hwang, A. Toropov, D.  
927 Leszczynska and J. Leszczynski. 2011. Using nano-QSAR to predict the cytotoxicity of metal oxide  
928 nanoparticles. *Nature Nanotechnology* 6:175.
- 929 C. D. Walkey, J. B. Olsen, F. Song, R. Liu, H. Guo, D. W. H. Olsen, Y. Cohen, A. Emili and W. C. W.  
930 Chan. 2014. Protein Corona Fingerprinting Predicts the Cellular Interaction of Gold and Silver  
931 Nanoparticles. *ACS Nano* 8:2439-2455.
- 932 D. E. Jones, H. Ghandehari and J. C. Facelli. 2015. Predicting cytotoxicity of PAMAM dendrimers  
933 using molecular descriptors. *Beilstein journal of nanotechnology* 6:1886-1896.
- 934 H. C. Bygd, K. D. Forsmark and K. M. Bratlie. 2015. Altering in vivo macrophage responses with  
935 modified polymer properties. *Biomaterials* 56:187-197.
- 936 H. Baharifar and A. Amani. 2016. Cytotoxicity of chitosan/streptokinase nanoparticles as a function of  
937 size: An artificial neural networks study. *Nanomedicine: Nanotechnology, Biology and Medicine*  
938 12:171-180.
- 939 M. Bilal, E. Oh, R. Liu, J. C. Breger, I. L. Medintz and Y. Cohen. 2019. Bayesian Network Resource  
940 for Meta-Analysis: Cellular Toxicity of Quantum Dots. *Small* 0:1900510.
- 941 E. Oh, R. Liu, A. Nel, K. B. Gemill, M. Bilal, Y. Cohen and I. L. Medintz. 2016. Meta-analysis of  
942 cellular toxicity for cadmium-containing quantum dots. *Nature Nanotechnology* 11:479.

- 943 E. Casals, M. F. Gusta, J. Piella, G. Casals, W. Jiménez and V. Puentes. 2017. Intrinsic and Extrinsic  
944 Properties Affecting Innate Immune Responses to Nanoparticles: The Case of Cerium Oxide. *Front*  
945 *Immunol* 8:970-970.
- 946 E. Papa, J. P. Doucet, A. Sangion and A. Doucet-Panaye. 2016. Investigation of the influence of protein  
947 corona composition on gold nanoparticle bioactivity using machine learning approaches. *SAR and*  
948 *QSAR in Environmental Research* 27:521-538.
- 949 R. Liu, W. Jiang, C. D. Walkey, W. C. W. Chan and Y. Cohen. 2015. Prediction of nanoparticles-cell  
950 association based on corona proteins and physicochemical properties. *Nanoscale* 7:9664-9675.
- 951 Z. Ban, Q. Zhou, A. Sun, L. Mu and X. Hu. 2018. Screening Priority Factors Determining and Predicting  
952 the Reproductive Toxicity of Various Nanoparticles. *Environmental Science & Technology* 52:9666-  
953 9676.
- 954 J. M. Gernand and E. A. Casman. 2014. A Meta-Analysis of Carbon Nanotube Pulmonary Toxicity  
955 Studies—How Physical Dimensions and Impurities Affect the Toxicity of Carbon Nanotubes. *Risk*  
956 *Analysis* 34:583-597.
- 957 X. Liu, K. Tang, S. Harper, B. Harper, J. A. Steevens and R. Xu. 2013. Predictive modeling of  
958 nanomaterial exposure effects in biological systems. *International journal of nanomedicine* 8 Suppl  
959 1:31-43.
- 960 V. V. Kleandrova, F. Luan, H. González-Díaz, J. M. Ruso, A. Speck-Planche and M. N. D. S. Cordeiro.  
961 2014b. Computational Tool for Risk Assessment of Nanomaterials: Novel QSTR-Perturbation Model  
962 for Simultaneous Prediction of Ecotoxicity and Cytotoxicity of Uncoated and Coated Nanoparticles  
963 under Multiple Experimental Conditions. *Environmental Science & Technology* 48:14686-14694.
- 964 V. Kovalishyn, N. Abramenko, I. Kopernyk, L. Charochkina, L. Metelytsia, I. V. Tetko, W. Peijnenburg  
965 and L. Kustov. 2018. Modelling the toxicity of a large set of metal and metal oxide nanoparticles using  
966 the OCHEM platform. *Food and chemical toxicology : an international journal published for the British*  
967 *Industrial Biological Research Association* 112:507-517.
- 968 G. Basei, D. Hristozov, L. Lamon, A. Zabeo, N. Jeliaskova, G. Tsiliki, A. Marcomini and A. Torsello.  
969 2019. Making use of available and emerging data to predict the hazards of engineered nanomaterials by  
970 means of in silico tools: A critical review. *NanoImpact* 13:76-99.
- 971 V. Christen, M. Camenzind and K. Fent. 2014. Silica nanoparticles induce endoplasmic reticulum stress  
972 response, oxidative stress and activate the mitogen-activated protein kinase (MAPK) signaling pathway.  
973 *Toxicology Reports* 1:1143-1151.
- 974 N. Sizochenko, B. Rasulev, A. Gajewicz, V. Kuz'min, T. Puzyn and J. Leszczynski. 2014. From basic  
975 physics to mechanisms of toxicity: the “liquid drop” approach applied to develop predictive  
976 classification models for toxicity of metal oxide nanoparticles. *Nanoscale* 6:13986-13993.
- 977 F. Gharagheizi and R. F. Alamdari. 2008. A Molecular-Based Model for Prediction of Solubility of C60  
978 Fullerene in Various Solvents. *Fullerenes, Nanotubes and Carbon Nanostructures* 16:40-57.
- 979 A. Gerber, M. Bundschuh, D. Klingelhofer and D. A. Groneberg. 2013. Gold nanoparticles: recent  
980 aspects for human toxicology. *Journal of Occupational Medicine and Toxicology (London, England)*  
981 8:32-32.
- 982 T. Petrova, B. F. Rasulev, A. A. Toropov, D. Leszczynska and J. Leszczynski. 2011. Improved model  
983 for fullerene C60 solubility in organic solvents based on quantum-chemical and topological descriptors.  
984 *Journal of Nanoparticle Research* 13:3235-3247.

- 985 C. Oksel, C. Y. Ma, J. J. Liu, T. Wilkins and X. Z. Wang. (2017). Literature Review of (Q)SAR  
986 Modelling of Nanomaterial Toxicity. In: L. Tran, M. A. Bañares and R. Rallo eds. *Modelling the*  
987 *Toxicity of Nanoparticles*. Cham: Springer International Publishing, 103-142.
- 988 J. J. Villaverde, B. Sevilla-Morán, C. López-Goti, J. L. Alonso-Prados and P. Sandín-España. 2018.  
989 Considerations of nano-QSAR/QSPR models for nanopesticide risk assessment within the European  
990 legislative framework. *Science of The Total Environment* 634:1530-1539.
- 991 K. Pathakoti, M.-J. Huang, J. D. Watts, X. He and H.-M. Hwang. 2014. Using experimental data of  
992 *Escherichia coli* to develop a QSAR model for predicting the photo-induced cytotoxicity of metal oxide  
993 nanoparticles. *Journal of Photochemistry and Photobiology B: Biology* 130:234-240.
- 994 J. Nikota, A. Williams, C. L. Yauk, H. Wallin, U. Vogel and S. Halappanavar. 2016. Meta-analysis of  
995 transcriptomic responses as a means to identify pulmonary disease outcomes for engineered  
996 nanomaterials. *Particle and Fibre Toxicology* 13:25.
- 997 K. Jagiello, M. Grzonkowska, M. Swirog, L. Ahmed, B. Rasulev, A. Avramopoulos, M. G.  
998 Papadopoulos, J. Leszczynski and T. Puzyn. 2016. Advantages and limitations of classic and 3D QSAR  
999 approaches in nano-QSAR studies based on biological activity of fullerene derivatives. *Journal of*  
1000 *Nanoparticle Research* 18:256.
- 1001 V. V. Kleandrova, F. Luan, H. González-Díaz, J. M. Ruso, A. Melo, A. Speck-Planche and M. N. D. S.  
1002 Cordeiro. 2014. Computational ecotoxicology: Simultaneous prediction of ecotoxic effects of  
1003 nanoparticles under different experimental conditions. *Environment International* 73:288-294.
- 1004 A. Bigdeli, M. R. Hormozi-Nezhad and H. Parastar. 2015. Using nano-QSAR to determine the most  
1005 responsible factor(s) in gold nanoparticle exocytosis. *RSC Advances* 5:57030-57037.
- 1006 A. Mikolajczyk, A. Gajewicz, B. Rasulev, N. Schaeublin, E. Maurer-Gardner, S. Hussain, J.  
1007 Leszczynski and T. Puzyn. 2015. Zeta Potential for Metal Oxide Nanoparticles: A Predictive Model  
1008 Developed by a Nano-Quantitative Structure–Property Relationship Approach. *Chemistry of Materials*  
1009 27:2400-2407.
- 1010 M. González-Durruthy, L. C. Alberici, C. Curti, Z. Naal, D. T. Atique-Sawazaki, J. M. Vázquez-Naya,  
1011 H. González-Díaz and C. R. Munteanu. 2017. Experimental–Computational Study of Carbon Nanotube  
1012 Effects on Mitochondrial Respiration: In Silico Nano-QSPR Machine Learning Models Based on New  
1013 Raman Spectra Transform with Markov–Shannon Entropy Invariants. *Journal of Chemical Information*  
1014 *and Modeling* 57:1029-1044.
- 1015 OECD. (2004). *Guidance Document on the Validation of (Quantitative) Structure-Activity Relationship*  
1016 *[(Q)SAR] Models*. OECD Series on Testing and Assessment.
- 1017 F. Zhao, Y. Zhao, Y. Liu, X. Chang, C. Chen and Y. Zhao. 2011. Cellular Uptake, Intracellular  
1018 Trafficking, and Cytotoxicity of Nanomaterials. *Small* 7:1322-1337.
- 1019 S. Elmore. 2007. Apoptosis: a review of programmed cell death. *Toxicol Pathol* 35:495-516.
- 1020 A. Gajewicz, T. Puzyn, K. Odziomek, P. Urbaszek, A. Haase, C. Riebeling, A. Luch, M. A. Irfan, R.  
1021 Landsiedel, M. van der Zande and H. Bouwmeester. 2018. Decision tree models to classify  
1022 nanomaterials according to the DF4nanoGrouping scheme. *Nanotoxicology* 12:1-17.
- 1023 C. Oksel, D. A. Winkler, C. Y. Ma, T. Wilkins and X. Z. Wang. 2016. Accurate and interpretable  
1024 nanoSAR models from genetic programming-based decision tree construction approaches.  
1025 *Nanotoxicology* 10:1001-1012.

- 1026 A. A. Toropov and A. P. Toropova. 2014. Optimal descriptor as a translator of eclectic data into endpoint  
1027 prediction: Mutagenicity of fullerene as a mathematical function of conditions. *Chemosphere* 104:262-  
1028 264.
- 1029 A. A. Toropov and A. P. Toropova. 2015. Quasi-SMILES and nano-QFAR: United model for  
1030 mutagenicity of fullerene and MWCNT under different conditions. *Chemosphere* 139:18-22.
- 1031 J. Brownlee. (2013). A Tour of Machine Learning Algorithms. Journal [Online] Available at:  
1032 <http://machinelearningmastery.com/a-tour-of-machine-learning-algorithms/>. Accessed on September  
1033 2019
- 1034 R. Concu, V. V. Kleandrova, A. Speck-Planche and M. N. D. S. Cordeiro. 2017. Probing the toxicity of  
1035 nanoparticles: a unified in silico machine learning model based on perturbation theory. *Nanotoxicology*  
1036 11:891-906.
- 1037 V. V. Kleandrova, A. Speck-Planche and M. N. D. S. Cordeiro. 2017. Probing the toxicity of  
1038 nanoparticles: a unified in silico machine learning model based on perturbation theory AU - Concu,  
1039 Riccardo. *Nanotoxicology* 11:891-906.
- 1040 F. Luan, V. V. Kleandrova, H. González-Díaz, J. M. Ruso, A. Melo, A. Speck-Planche and M. N. D. S.  
1041 Cordeiro. 2014. Computer-aided nanotoxicology: assessing cytotoxicity of nanoparticles under diverse  
1042 experimental conditions by using a novel QSTR-perturbation approach. *Nanoscale* 6:10623-10630.
- 1043 S. George, T. Xia, R. Rallo, Y. Zhao, Z. Ji, S. Lin, X. Wang, H. Zhang, B. France, D. Schoenfeld, R.  
1044 Damoiseaux, R. Liu, S. Lin, K. A. Bradley, Y. Cohen and A. E. Nel. 2011. Use of a High-Throughput  
1045 Screening Approach Coupled with In Vivo Zebrafish Embryo Screening To Develop Hazard Ranking  
1046 for Engineered Nanomaterials. *ACS Nano* 5:1805-1817.
- 1047 A. Speck-Planche, V. V. Kleandrova, F. Luan and M. N. Cordeiro. 2015. Computational modeling in  
1048 nanomedicine: prediction of multiple antibacterial profiles of nanoparticles using a quantitative  
1049 structure-activity relationship perturbation model. *Nanomedicine (London, England)* 10:193-204.
- 1050 F. Murphy, B. Sheehan, M. Mullins, H. Bouwmeester, H. J. P. Marvin, Y. Bouzembrak, A. L. Costa, R.  
1051 Das, V. Stone and S. A. M. Tofail. 2016. A Tractable Method for Measuring Nanomaterial Risk Using  
1052 Bayesian Networks. *Nanoscale research letters* 11:503-503.
- 1053 H. J. P. Marvin, Y. Bouzembrak, E. M. Janssen, M. van der Zande, F. Murphy, B. Sheehan, M. Mullins  
1054 and H. Bouwmeester. 2017. Application of Bayesian networks for hazard ranking of nanomaterials to  
1055 support human health risk assessment. *Nanotoxicology* 11:123-133.
- 1056 B. Harper, D. Thomas, S. Chikkagoudar, N. Baker, K. Tang, A. Heredia-Langner, R. Lins and S. Harper.  
1057 2015. Comparative hazard analysis and toxicological modeling of diverse nanomaterials using the  
1058 embryonic zebrafish (EZ) metric of toxicity. *Journal of Nanoparticle Research* 17:250.
- 1059 C.-Y. Shao, S.-Z. Chen, B.-H. Su, Y. J. Tseng, E. X. Esposito and A. J. Hopfinger. 2013. Dependence  
1060 of QSAR Models on the Selection of Trial Descriptor Sets: A Demonstration Using Nanotoxicity  
1061 Endpoints of Decorated Nanotubes. *Journal of Chemical Information and Modeling* 53:142-158.
- 1062 H. Zhang, Z. Ji, T. Xia, H. Meng, C. Low-Kam, R. Liu, S. Pokhrel, S. Lin, X. Wang, Y.-P. Liao, M.  
1063 Wang, L. Li, R. Rallo, R. Damoiseaux, D. Telesca, L. Mädler, Y. Cohen, J. I. Zink and A. E. Nel. 2012.  
1064 Use of Metal Oxide Nanoparticle Band Gap To Develop a Predictive Paradigm for Oxidative Stress and  
1065 Acute Pulmonary Inflammation. *ACS Nano* 6:4349-4368.
- 1066 D. Fourches, D. Pu, C. Tassa, R. Weissleder, S. Y. Shaw, R. J. Mumper and A. Tropsha. 2010.  
1067 Quantitative Nanostructure–Activity Relationship Modeling. *ACS Nano* 4:5703-5712.

- 1068 N. Sizochenko, A. Mikolajczyk, K. Jagiello, T. Puzyn, J. Leszczynski and B. Rasulev. 2018. How the  
1069 toxicity of nanomaterials towards different species could be simultaneously evaluated: a novel multi-  
1070 nano-read-across approach. *Nanoscale* 10:582-591.
- 1071 D. A. Winkler, F. R. Burden, B. Yan, R. Weissleder, C. Tassa, S. Shaw and V. C. Epa. 2014. Modelling  
1072 and predicting the biological effects of nanomaterials. *SAR and QSAR in Environmental Research*  
1073 25:161-172.
- 1074 V. C. Epa, F. R. Burden, C. Tassa, R. Weissleder, S. Shaw and D. A. Winkler. 2012. Modeling  
1075 Biological Activities of Nanoparticles. *Nano Letters* 12:5808-5812.
- 1076 S. Y. Shaw, E. C. Westly, M. J. Pittet, A. Subramanian, S. L. Schreiber and R. Weissleder. 2008.  
1077 Perturbational profiling of nanomaterial biologic activity. *Proceedings of the National Academy of*  
1078 *Sciences* 105:7387-7392.
- 1079 Y. T. Chau and C. W. Yap. 2012. Quantitative Nanostructure–Activity Relationship modelling of  
1080 nanoparticles. *RSC Advances* 2:8489-8496.
- 1081 K. P. Singh and S. Gupta. 2014. Nano-QSAR modeling for predicting biological activity of diverse  
1082 nanomaterials. *RSC Advances* 4:13215-13230.
- 1083 M. Ghorbanzadeh, M. H. Fatemi and M. Karimpour. 2012. Modeling the Cellular Uptake of  
1084 Magnetofluorescent Nanoparticles in Pancreatic Cancer Cells: A Quantitative Structure Activity  
1085 Relationship Study. *Industrial & Engineering Chemistry Research* 51:10712-10718.
- 1086 A. A. Toropov, A. P. Toropova, T. Puzyn, E. Benfenati, G. Gini, D. Leszczynska and J. Leszczynski.  
1087 2013. QSAR as a random event: Modeling of nanoparticles uptake in PaCa2 cancer cells. *Chemosphere*  
1088 92:31-37.
- 1089 G. Melagraki and A. Afantitis. 2014. Enalos InSilicoNano platform: an online decision support tool for  
1090 the design and virtual screening of nanoparticles. *RSC Advances* 4:50713-50725.
- 1091 T. C. Le, H. Yin, R. Chen, Y. Chen, L. Zhao, P. S. Casey, C. Chen and D. A. Winkler. 2016. An  
1092 Experimental and Computational Approach to the Development of ZnO Nanoparticles that are Safe by  
1093 Design. *Small* 12:3568-3577.
- 1094 R. Liu, R. Rallo, S. George, Z. Ji, S. Nair, A. E. Nel and Y. Cohen. 2011. Classification NanoSAR  
1095 Development for Cytotoxicity of Metal Oxide Nanoparticles. *Small* 7:1118-1126.
- 1096 A. P. Toropova, A. A. Toropov, E. Benfenati, R. Korenstein, D. Leszczynska and J. Leszczynski. 2015b.  
1097 Optimal nano-descriptors as translators of eclectic data into prediction of the cell membrane damage by  
1098 means of nano metal-oxides. *Environmental science and pollution research international* 22:745-757.
- 1099 T. Patel, D. Telesca, C. Low-Kam, Z. Ji, H. Zhang, T. Xia, J. I. Zinc and A. E. Nel. 2014. Relating  
1100 Nanoparticle Properties to Biological Outcomes in Exposure Escalation Experiments. *Environmetrics*  
1101 25:57-68.
- 1102 C. Sayes and I. Ivanov. 2010. Comparative Study of Predictive Computational Models for Nanoparticle-  
1103 Induced Cytotoxicity. *Risk Analysis* 30:1723-1734.
- 1104 A. P. Toropova and A. A. Toropov. 2013. Optimal descriptor as a translator of eclectic information into  
1105 the prediction of membrane damage by means of various TiO<sub>2</sub> nanoparticles. *Chemosphere* 93:2650-  
1106 2655.

- 1107 E. Papa, J. P. Doucet and A. Doucet-Panaye. 2015. Linear and non-linear modelling of the cytotoxicity  
1108 of TiO<sub>2</sub> and ZnO nanoparticles by empirical descriptors. *SAR QSAR Environ Res* 26:647-665.
- 1109 T. X. Trinh, M. K. Ha, J. S. Choi, H. G. Byun and T. H. Yoon. 2018b. Curation of datasets, assessment  
1110 of their quality and completeness, and nanoSAR classification model development for metallic  
1111 nanoparticles. *Environmental Science: Nano* 5:1902-1910.
- 1112 T. X. Trinh, J. S. Choi, H. Jeon, H. G. Byun, T. H. Yoon and J. Kim. 2018. Quasi-SMILES-Based Nano-  
1113 Quantitative Structure-Activity Relationship Model to Predict the Cytotoxicity of Multiwalled Carbon  
1114 Nanotubes to Human Lung Cells. *Chemical research in toxicology* 31:183-190.
- 1115 J.-S. Choi, M. K. Ha, T. X. Trinh, T. H. Yoon and H.-G. Byun. 2018. Towards a generalized toxicity  
1116 prediction model for oxide nanomaterials using integrated data from different sources. *Scientific Reports*  
1117 8:6110.
- 1118 J.-S. Choi, T. X. Trinh, T.-H. Yoon, J. Kim and H.-G. Byun. 2019. Quasi-QSAR for predicting the cell  
1119 viability of human lung and skin cells exposed to different metal oxide nanomaterials. *Chemosphere*  
1120 217:243-249.
- 1121 X. Hu, S. Cook, P. Wang and H.-M. Hwang. 2009. In vitro evaluation of cytotoxicity of engineered  
1122 metal oxide nanoparticles. *Sci Total Environ* 407:3070-3072.
- 1123 G. Chen, W. J. G. M. Peijnenburg, V. Kovalishyn and M. G. Vijver. 2016. Development of  
1124 nanostructure–activity relationships assisting the nanomaterial hazard categorization for risk assessment  
1125 and regulatory decision-making. *RSC Advances* 6:52227-52235.
- 1126 A. P. Toropova, A. A. Toropov and E. Benfenati. 2015. A quasi-QSPR modelling for the photocatalytic  
1127 decolourization rate constants and cellular viability (CV%) of nanoparticles by CORAL. *SAR QSAR*  
1128 *Environ Res* 26:29-40.
- 1129 A. Mikolajczyk, A. Gajewicz, E. Mulkiwicz, B. Rasulev, M. Marchelek, M. Diak, S. Hirano, A.  
1130 Zaleska-Medynska and T. Puzyn. 2018. Nano-QSAR modeling for ecosafe design of heterogeneous  
1131 TiO<sub>2</sub>-based nano-photocatalysts. *Environmental Science: Nano* 5:1150-1160.
- 1132 I. Furxhi, F. Murphy, M. Mullins and C. A. Poland. 2019a. Machine learning prediction of nanoparticle  
1133 in vitro toxicity: A comparative study of classifiers and ensemble-classifiers using the Copeland Index.  
1134 *Toxicology Letters* 312:157-166.
- 1135 I. Furxhi, F. Murphy, C. A. Poland, B. Sheehan, M. Mullins and P. Mantecca. 2019b. Application of  
1136 Bayesian networks in determining nanoparticle-induced cellular outcomes using transcriptomics.  
1137 *Nanotoxicology* 13:827-848.
- 1138 A. Tamvakis, C.-N. Anagnostopoulos, G. Tsirtsis, A. D. Niros and S. Spatharis. 2018. Optimized  
1139 Classification Predictions with a New Index Combining Machine Learning Algorithms. *International*  
1140 *Journal on Artificial Intelligence Tools* 27:1850012.
- 1141 G. Tsiliki, C. R. Munteanu, J. A. Seoane, C. Fernandez-Lozano, H. Sarimveis and E. L. Willighagen.  
1142 2015. RRegrs: an R package for computer-aided model selection with multiple regression models.  
1143 *Journal of Cheminformatics* 7:46.
- 1144 C. Helma, M. Rautenberg and D. Gebele. 2017. Nano-Lazar: Read across Predictions for Nanoparticle  
1145 Toxicities with Calculated and Measured Properties. *Frontiers in Pharmacology* 8.

- 1146 M. E. Vance, T. Kuiken, E. P. Vejerano, S. P. McGinnis, M. F. Hochella, Jr., D. Rejeski and M. S. Hull.  
1147 2015. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory.  
1148 Beilstein journal of nanotechnology 6:1769-1780.
- 1149 C. Oksel, C. Y. Ma, J. J. Liu, T. Wilkins and X. Z. Wang. 2015b. (Q)SAR modelling of nanomaterial  
1150 toxicity: A critical review. Particuology 21:1-19.
- 1151 A. R. Ribeiro, P. E. Leite, P. Falagan-Lotsch, F. Benetti, C. Micheletti, H. C. Budtz, N. R. Jacobsen, P.  
1152 N. Lisboa-Filho, L. A. Rocha, D. Kühnel, D. Hristozov and J. M. Granjeiro. 2017. Challenges on the  
1153 toxicological predictions of engineered nanoparticles. NanoImpact 8:59-72.
- 1154 L. P. W. Clausen and S. F. Hansen. 2018. The ten decrees of nanomaterials regulations. Nature  
1155 Nanotechnology 13:766-768.
- 1156 N. Basant and S. Gupta. 2017. Multi-target QSTR modeling for simultaneous prediction of multiple  
1157 toxicity endpoints of nano-metal oxides. Nanotoxicology 11:339-350.
- 1158 P. De, S. Kar, K. Roy and J. Leszczynski. 2018. Second generation periodic table-based descriptors to  
1159 encode toxicity of metal oxide nanoparticles to multiple species: QSTR modeling for exploration of  
1160 toxicity mechanisms. Environmental Science: Nano 5:2742-2760.
- 1161 I. Furxhi, F. Murphy, B. Sheehan, M. Mullins and P. Mantecca. (2018). Predicting Nanomaterials  
1162 toxicity pathways based on genome-wide transcriptomics studies using Bayesian networks. 2018 IEEE  
1163 18th International Conference on Nanotechnology (IEEE-NANO). 1-4.
- 1164 K. Donaldson, A. Schinwald, F. Murphy, W.-S. Cho, R. Duffin, L. Tran and C. Poland. 2013. The  
1165 Biologically Effective Dose in Inhalation Nanotoxicology. Accounts of Chemical Research 46:723-732.
- 1166 C. A. Poland, M. R. Miller, R. Duffin and F. Cassee. 2014. The elephant in the room: reproducibility in  
1167 toxicology. Particle and Fibre Toxicology 11:42.
- 1168 T. F. França and J. M. Monserrat. 2018. Reproducibility crisis in science or unrealistic expectations?  
1169 EMBO reports 19:e46008.
- 1170 C. O. Hendren, C. M. Powers, M. D. Hoover and S. L. Harper. 2015. The Nanomaterial Data Curation  
1171 Initiative: A collaborative approach to assessing, evaluating, and advancing the state of the field.  
1172 Beilstein Journal of Nanotechnology 6:1752-1762.
- 1173 C. M. Powers, K. A. Mills, S. A. Morris, F. Klaessig, S. Gaheen, N. Lewinski and C. Ogilvie Hendren.  
1174 2015. Nanocuration workflows: Establishing best practices for identifying, inputting, and sharing data  
1175 to inform decisions on nanomaterials. Beilstein journal of nanotechnology 6:1860-1871.
- 1176 C. O. P. Hendren, C. M.; Hoover, M. D.; Harper, S. L. 2015. The Nanomaterial Data Curation Initiative:  
1177 A collaborative approach to assessing, evaluating, and advancing the state of the field. Beilstein J  
1178 Nanotechnol 6:1752–1762.
- 1179 S. Karcher, E. L. Willighagen, J. Rumble, F. Ehrhart, C. T. Evelo, M. Fritts, S. Gaheen, S. L. Harper,  
1180 M. D. Hoover, N. Jeliaskova, N. Lewinski, R. L. Marchese Robinson, K. C. Mills, A. P. Mustad, D. G.  
1181 Thomas, G. Tsiliki and C. O. Hendren. 2018. Integration among databases and data sets to support  
1182 productive nanotechnology: Challenges and recommendations. NanoImpact 9:85-101.
- 1183 J. T. K. Quik, M. Bakker, D. van de Meent, M. Poikkimäki, M. Dal Maso and W. Peijnenburg. 2018.  
1184 Directions in QPPR development to complement the predictive models used in risk assessment of  
1185 nanomaterials. NanoImpact 11:58-66.

- 1186 D. G. Thomas, S. Gaheen, S. L. Harper, M. Fritts, F. Klaessig, E. Hahn-Dantona, D. Paik, S. Pan, G. A.  
1187 Stafford, E. T. Freund, J. D. Klemm and N. A. Baker. 2013. ISA-TAB-Nano: a specification for sharing  
1188 nanomaterial research data in spreadsheet-based format. *BMC biotechnology* 13:2.
- 1189 R. L. Marchese Robinson, M. T. D. Cronin, A.-N. Richarz and R. Rallo. 2015. An ISA-TAB-Nano based  
1190 data collection framework to support data-driven modelling of nanotoxicology. *Beilstein journal of*  
1191 *nanotechnology* 6:1978-1999.
- 1192 M. Córdoba and A. Zambon. 2017. How to handle nanomaterials? The re-entry of individuals into the  
1193 philosophy of chemistry. *Foundations of Chemistry* 19:185-196.
- 1194 S. Karcher, E. L. Willighagen, J. Rumble, F. Ehrhart, C. T. Evelo, M. Fritts, S. Gaheen, S. L. Harper,  
1195 M. D. Hoover, N. Jeliakova, N. Lewinski, R. L. Marchese Robinson, K. C. Mills, A. P. Mustad, D. G.  
1196 Thomas, G. Tsiliki and C. Ogilvie Hendren. 2018. Integration among databases and data sets to support  
1197 productive nanotechnology: Challenges and recommendations. *NanoImpact* 9:85-101.
- 1198 B. Saini and S. Srivastava. 2018. Nanotoxicity prediction using computational modelling - review and  
1199 future directions. *IOP Conference Series: Materials Science and Engineering* 348:012005.
- 1200 M. K. Ha, T. X. Trinh, J. S. Choi, D. Maulina, H. G. Byun and T. H. Yoon. 2018. Toxicity Classification  
1201 of Oxide Nanomaterials: Effects of Data Gap Filling and PChem Score-based Screening Approaches.  
1202 *Scientific Reports* 8:3141.
- 1203 P. Grodzinski, C. H. Liu, C. M. Hartshorn, S. A. Morris and L. M. Russell. 2019. NCI Alliance for  
1204 Nanotechnology in Cancer – from academic research to clinical interventions. *Biomedical Microdevices*  
1205 21:32.  
1206
- 1207
- 1208