PATROLS
Advanced Tools for NanoSafety Testing

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PATROLS Standard Operating Procedures (SOP)

QSAR modelling of environmentally relevant fate and effect endpoints of nanomaterials

This is a SOP recommended for external use by PATROLS

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This SOP is based on the modelling activities performed within WP6 of the PATROLS project, in particular on the development of a QSAR for predicting the response of *Daphnia magna* following exposure to metallic nanomaterials. The same approaches as described in this SOP can be used for other environmentally relevant fate and effect related endpoints of nanomaterials.

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1 Introduction:

DOMAIN: Modelling environmental fate and effects of nanomaterials

1.1 General

Proper risk assessment of nanomaterials requires the generation of various kinds of data on endpoints relevant for the assessment of the fate and effects of the nanomaterials. As for all chemicals, both fate processes and adverse effects of nanomaterials are dependent not only on the intrinsic properties of the nanomaterial but also on the composition of the exposure medium as the latter affects the extrinsic properties of any nanomaterial. Intrinsic properties of relevance include in this respect for instance the particle size, surface coating, shape, and chemical composition. Extrinsic properties of interest include surface charge and reactivity

(Peijnenburg et al., 2015), with both properties affecting the fate as well as the effects of nanomaterials. Generation of the minimum set of test needed for proper risk assessment requires intensive experimentation. Within REACH the extent of data needed increases upon increasing production and use volumes of the materials, thus requiring increasing efforts and costs. Thereupon, considerations of animal welfare require that effect testing is kept to a minimum in view of the need of being in compliance with the 3Rs (Replacement, Reduction and Refinement) principles, as embraced by the scientific and policy communities dealing with Nanosafety.

Properly validated predictive models are one of the means of minimizing animal testing and reducing testing costs. The models build upon the basic assumption that structural properties that are directly or indirectly related to the mechanism of action underlying a specific fate or effect endpoint will allow for the development of predictive *in silico* models that can be used to quantify this specific fate or effect endpoint for hitherto untested nanomaterials. Application of such models not only contributes to the reduction of the number of experimental tests to be performed, but the models can for instance also be applied for other purposes like the identification of nanomaterials that are less harmful and hence safer-by-design than one or more of their structural counterparts. Models that predict the fate and toxicity of nanomaterials on the basis of their physicochemical properties are called Nano-QSAR models.

The OECD has established principles for the validation for regulatory purposes of (Q)SAR models as summarized in a guidance document (OECD, 2007). These principles constitute the following:

“To facilitate the consideration of a (Q)SAR model for regulatory purposes, it should be associated with the following information:

1. A defined endpoint;
2. An unambiguous algorithm;
3. A defined domain of applicability;
4. Appropriate measures of goodness-of-fit, robustness and predictivity;
5. A mechanistic interpretation, if possible.”

This SOP is focussed on deriving a QSAR model suited for predicting the dose-response relationship of metallic nanomaterials, on the basis of the OECD guidelines for the validation of QSAR models for regulatory purposes. Hence, the approach used here is not restricted to this specific example but can be used for other environmental fate and effect related endpoints as well. The main aim of the SOP is to systematically address the various steps that need to be taken for proper model development and model validation according to the broadly accepted approaches agreed upon within OECD.

1.2 Scope and limits of the protocol

The scope of the SOP is in itself restricted to predicting adverse responses of the waterflea *Daphnia magna* to metallic nanoparticles. In this specific case the application is basically the prediction of the value of the Hill-coefficient which is the key parameter in determining the dose-response curve of any chemical for a specific endpoint. The Hill coefficient, n_H, i.e. the steepness and shape of the response curve was introduced as an empirical description by

Hill (1910), and is typically used to quantify the response of a receptor to a stressor (Goldbeter and Dupont 1990). It is a sigmoidal function commonly prescribed in the OECD guidance document on testing chemicals and nanomaterials (OECD 2020). The Hill equation is quite often utilized to predict the dose–response relationship of nanomaterials to a variety of different organisms and different metal-based nanomaterials:

$$E = \frac{100}{1+\left(\frac{EC_{50}}{[A]}\right)^{nH}} \quad (1)$$

The Hill equation requires only 3 parameters to determine the dose–response relationship. In the equation, n_H may be considered as the parameter that is dependent on the characteristics of the ENMs to which the biota are exposed. E is the magnitude of the response, [A] is the number concentration of ENM particle (# particles/L), EC_{50} is the number of ENMs particle (# particles/L) that produces 50% response, and n_H in the Hill coefficient. The values of n_H used in the Monte Carlo optimization were obtained from fitting dose-response relationships for 11 metal-based nanomaterials (60 datasets, number of individual observations: *n* = 367) from 20 reports. The data used for model derivation were obtained in media of different composition and media composition was included in the modelling.

As the dataset used to generate the model is restricted to metallic nanomaterials, the model is applicable only for this class of ENMs. Fully similar approaches may be taken to develop similar models for additional classes of ENMs. For reliable predictions using the developed model, it is essential to determine on forehand whether the metallic nanomaterial belongs to its applicability domain. The presented model is presented in detail in D6.4 of the PATROLS project.

2 Terms and Definitions:

Nanoscale
Length range in between 1 nm to 100 nm.

Nanotechnology
Application of scientific knowledge to manipulate and control matter predominantly in the *nanoscale* to make use of size- and structure-dependent properties and phenomena distinct from those associated with individual atoms or molecules, or extrapolation from larger sizes of the same material.

Nanomaterial
Material with any external dimension in the *nanoscale* or having internal structure or surface structure in the nanoscale.

Engineered nanomaterial
Nanomaterial designed for specific purpose or function.

Nano-QSAR

Method for modelling the relationships between properties of a nanomaterial and either its fate in a medium or its toxic response.

Training dataset

A dataset used for the development of a model.

Validation dataset

A dataset used for the calibration of a model.

3 Abbreviations:

ENM: Engineered NanoMaterial

MLR – Multiple Linear Regression

Nano-QSAR – QSAR model for nanomaterials

OECD – Organisation for Economic Cooperation and Development

QSAR – Quantitative Structure-Activity Relationship

$\mathbf{R^2}$ – determination coefficient

$\mathbf{RMSE_C}$ – root mean square error of calibration

$\mathbf{Q^2_{CV}}$ – cross-validated correlation coefficient

$\mathbf{RMSE_{CV}}$ – cross-validated root mean square error of prediction

$\mathbf{Q_{2EXT(F2)}}$ – the externally validated determination coefficient

$\mathbf{RMSE_{EXT}}$ – the root mean square error of prediction

4 Principle of the Method:

The protocol is based on the implementation of the OECD principles for the validation for regulatory purposes of (Q)SAR models. In this specific case a quasi-QSAR model was developed for predicting the values of the Hill-coefficient. In a quasi-QSAR model the physicochemical property of nanomaterials and the exposure conditions are represented by quasi-SMILES. Quasi-SMILES represents eclectic data related to an endpoint, where eclectic data include all conditions (controlled or observed) that affect the experiment results (Bragazzi et al., 2016). In this specific case, the composition of the medium was included in the quasi-SMILES.

The values of n_H, used in the Monte Carlo optimization that is the basic method applied, were obtained from fitting dose-response relationships for 11 metal-based nanomaterials (60

datasets, number of individual observations: n = 367) from 20 literature reports. Validation of the model obtained was performed by means of establishing a randomly and independently selected set of experimental data, derived from 15 datasets reported in literature with 72 individual responses of *Daphnia magna* to metallic nanomaterials as obtained from 10 literature reports.

5 Description of the Method:

5.1 Biological setting & test system used:

The biological test system of relevance was the water flea *Dapnia magna*. The endpoint of assessment was immobilization. Dose-response data were obtained for metallic nanomaterials.

The Quasi-QSAR model was developed with the so-called quasi-SMILES approach (Toropova et al. 2011). Quasi-SMILES is a string using physicochemical features and/or biochemical conditions as a replacement to conventional SMILES. The quasi-SMILES-based QSAR model can be demonstrated by the following equation:

$$n_H = C0 + C1 \times DCW(T, N_{Epoch}) \quad (2)$$

Where $C0$ and $C1$ are the intercept and the slope.

The correlation weight of the descriptor (DCW) is computed using the following equation:

$$DCW(T, N_{Epoch}) = \alpha \sum CW(S_k) + \beta \sum CW(SS_k) + \gamma \cdot CW(NOSP) \quad (3)$$

where S_k and SS_k are SMILES attributes which contain one- and two- SMILES elements respectively; $CW(S_k)$ and $CW(SS_k)$ are the correlation weights of the SMILES attributes; NOSP is an index which represents the presence or absence of chemical elements, i.e. nitrogen, oxygen, sulfur, and phosphorus; α, β, and γ are coefficients that can be 1 or 0, with 1 indicating that the SMILES attribute is involved in the calculation of the $DCW(T, N_{Epoch})$ whereas 0 indicates that the SMILES attribute is not involved. The $DCW(T, N_{Epoch})$ i.e., combinations of these values, present the probability to define diverse versions of the SMILES-based optimal descriptor. Note that the threshold (T) and the number of epochs (N_{Epoch}) are parameters of the optimisation that set the preferred statistical quality of the training set. The CORAL software (http://www.insilico.eu/coral) was employed to develop and validate the quasi-QSAR models.

The Quasi-SMILES employed in this study was composed of three components:

1 - one code for size of the ENMs. The code for ENMs size was assigned as a rounded number of nanoparticle diameter (nm) to an integer.

2 - one code for the test conditions and exposure duration and/or ENM coating material. The code for representing a toxic assay and/or ENMs coating was a combination of number and plus signs (i.e., 1+, 1++, 1+++, …), the numeral from 1 to 20 was used to represent the 20 reports listed in the Supplementary Tables S1 and S2, and the number of plus signs represents the number of variations of toxicity assay/ENMs coating in the report.

3 - one code for the ENM type.

A combination of number, symbol and SMILES was used to build up the quasi-QSAR string. The code for indicating the type of ENMs employed the SMILES line notation that was obtained from the PubChem online database.

Monte Carlo optimization, as based on repeated random sampling in the CORAL package, was employed to optimize the parameters being descriptive in the quasi-QSAR model and to make numerical estimations of unknown parameters. The number of random sampling within the Monte Carlo algorithm was set on a max of 60 datasets and the optimization of the quasi-QSAR string was reached based on R^2 of 0.8246. In this study, the optimization represented correlations between the fitted n_H and the quasi-QSAR string, which correlation weights is estimated as $\mathrm{DCW(T, N_{Epoch})}$ in equation (2). Additionally, the intercept ($\mathrm{C0}$) and slope ($\mathrm{C1}$) of the quasi-QSAR model in equation (2) are approximated by CORAL software.

5.2 Chemicals and reagents used:

Not applicable as this SOP refers to modelling existing data. It is to be noted that the chemical domain of the models developed is restricted to metallic ENMs whereas the endpoint of assessment in this specific case is immobilization of *Daphnia magna*.

5.3 Apparatus and equipment used:

Model development was performed using the CORAL software (http://www.insilico.eu/coral).

5.4 Reporting of protected elements:

Not applicable.

5.5 Health and safety precautions:

For the specific case of model development no specific precautions are of relevance, others than general issues related to modellers working in a safe and healthy personnel environment. Warranting such conditions is beyond the scope of this SOP.

5.6 Applicability:

The SOP is in itself applicable and has been demonstrated for the specific endpoint of impacts of ENMs on immobilization of *Daphnia magna*. The principles applied in this specific example are also valid for other fate and effect related endpoints.

5.7 Reagent preparation:

Not of relevance as this SOP deals with a modelling study: no experimental data are generated.

5.8 Procedure

It is to be stressed that this SOP builds upon following the OECD criteria for QSAR model development, as reported in paragraph 1.1:

1. A defined endpoint;
2. An unambiguous algorithm;
3. A defined domain of applicability;
4. Appropriate measures of goodness-of-fit, robustness and predictivity;
5. A mechanistic interpretation, if possible.

Each of these criteria will subsequently be dealt with to exemplify how they should be dealt with in model development for effect and fate related ENM endpoints.

5.8.1 A defined endpoint

As reported in paragraph 1.2, the endpoint of assessment is immobilization of *Daphnia magna* after exposure for in between 24 to 96 h (test duration was included as a modifier/descriptor in the Quasi-QSAR modelling). The tests with *Daphnia magna* are fully standardized within the OECD guidance documents (OECD no. 202) (OECD 2004) and modified for nanomaterials (the so-called NanoReg protocols) (Jantunen et al. 2018). The outcome is to determine the full dose–response relationship, describing the magnitude of the response as a function of exposure.

The actual endpoint of assessment is the Hill coefficient, as detailed in paragraph 1.2. This is a well-established endpoint that is suited for QSAR-modelling.

5.8.2 An unambiguous algorithm

The algorithm for prediction of the Hill coefficient was optimized by means of Monte Carlo optimization using CORAL software. This yielded an unambiguous algorithm that was subsequently validated by means of an independent dataset of values of the Hill coefficient as generated by employing the same testing protocols as used for the dataset used for model development.

5.8.3 A defined domain of applicability

The domain of applicability of the model developed is restricted to metallic nanomaterials. This applicability domain was operationalized by means of a literature search using the boolean operators: (TS= ("*toxicity" AND "effect*") AND TI=("nano") AND KP= (nano* AND metal* OR "nano* AND metal oxide*") AND KP=(crustacea* OR daphni*)) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article). Additionally, a literature search was done in Google Scholar using the following keywords: toxicity, effect, nano, metal, metal oxide, crustacea and daphni.

5.8.4 Appropriate measures of goodness-of-fit, robustness and predictivity

The goodness-of-fit was firstly determined on the basis of assessment of the relative error between experimental data and the model simulation/prediction was employed to illustrate the fit between the model and the experimental data. To this end the % sample deviation was calculation by means of equation 4:

$$\%\ \text{sample deviation (SDEV)} = \sqrt{\left[\frac{\sum\left(\frac{P_{\text{model calculation}} - P_{\text{experimental data}}}{P_{\text{experimental data}}}\right)^2}{N_{\text{data}} - 1}\right]} \times 100 \qquad (4)$$

where $P_{\text{model calculation}}$ and $P_{\text{experimental data}}$ are the log of toxicity value (# particles/L) determined from the simulation/prediction and the individual experimental response data for various magnitudes of the response, respectively, and N_{data} is the number of data. Model simulation/predictions include data restricted to the EC_5 - EC_{95} range because outside of this range the experimental noise is relative large, making the experimental data less robust for model building.

Figure 1 presents the comparison of model predictions and experimental data for both the test set and the validation set.

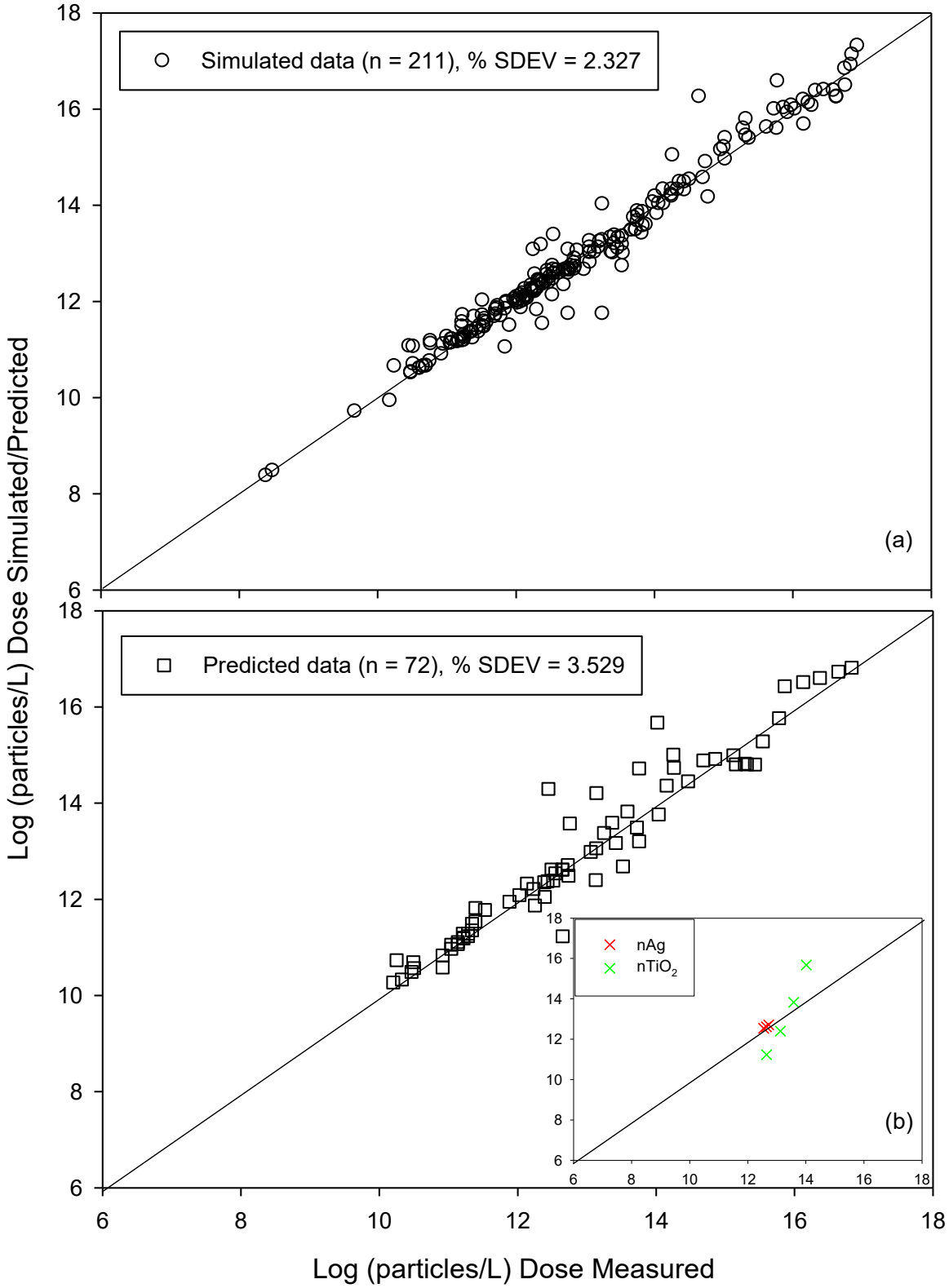

Figure 1. Comparison of dose-response between modelled and measured data for *Daphnia magna* immobilization, where (a) are the model simulations for 10 metal-based nanomaterials from 20 reports, and (b) are model predictions for 7 metal-based nanomaterials from 10 reports The inner figure in (b) is the best predicted dataset (% SDEV = 0.175) of polyethylene glycol capped nAg in Suwannee River dissolved organic carbon,

and the worst predicted case (% SDEV = 10.152) of $nTiO_2$ (Ø 20 nm) in 10-fold diluted ISO media under simulated solar radiation of 16:8 light-dark cycle.

The results presented in Figures 1a and 1 b demonstrate that the model is able to simulate and predict the experimental data well, with an overall SDEV of 2.7 % (R^2 of 0.958). The model performance is as good as model fitting shows that the % SDEV for the validation set is equal to 5.7 (R^2 of 0.937; *n* = 390 as the validation set includes all data in the EC_0 – EC_{100} range).

5.8.5 A mechanistic interpretation, if possible

Further research is needed on the actual mechanisms underpinning the experimental data. The set of metallic nanomaterials comprised dissolving ENMs (most notoriously: Ag, Cu, ZnO) as well as (very) sparingly soluble ENMs like TiO_2 and CeO_2.It is likely that in case of soluble ENMs release of metal ions and subsequent ion toxicity contributes to the immobilization of the daphnids. Thereupon, dissolution rates as well as ion reactivity are affected by the medium composition. No quantitative information is currently available to quantitatively include the impact of medium composition in the extent of adverse effect observed.

5.9 Quality control & acceptance criteria:

Figure 1 was used to depict the goodness of fit of the model obtained. The goodness of fit was shown for the test set used for model development and for the randomly selected validation set. As already indicated, it can be concluded from Figure 1 that the models are able to simulate and predict the experimental data well, with R^2 values equal to 0.958 for the test set and 0.937 for the validation set. The values of the SDEV of 2.7 % in case of the test set and 5.7 % for the validation set confirm the proper performance of the model.

6 Data Analysis and Reporting of Data:

The goodness-of-fit of the models were assessed using determination coefficient (R^2) and Root Mean Square Error of Calibration ($RMSE_C$):

$$R^2 = 1 - \frac{\sum_{i=1}^{n}\left(y_i^{obs} - y_i^{pred}\right)^2}{\sum_{i=1}^{n}\left(y_i^{obs} - \tilde{y}_i^{obs}\right)^2}$$

$$RMSE_C = \sqrt{\frac{\sum_{i=1}^{n}\left(y_i^{obs} - y_i^{pred}\right)^2}{n}}$$

where: y_i^{obs} – the experimentally (observed) value of the Hill coefficient for the i^{th} metallic nanoparticle; y_i^{pred} – the predicted value of the Hill coefficient for the i^{th} metallic nanoparticle; n – the number of nanoparticles in the training set.

7 Publications:

A manuscript on the model development is in preparation. Further details of the models developed can be found in D6.4 of the PATROLS project.

8 References

Bragazzi. N., A. Toropov, A. Toropova, E. Pechkova, C. Nicolini. Quasi-QSPR to predict proteins behavior under various concentrations of drug using nanoconductometric assay. NanoWorld Journal, 2, 71-77, 2016.

Goldbeter, A., Dupont, G. Allosteric regulation, cooperativity, and biochemical oscillations. Biophysical chemistry 37(1-3), 341-353, 1990.

Hill, A.V. The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. J Physiol (Lond) 40, 4-7, 1910.

Jantunen, A.P.K., Gottardo, S., Rasmussen, K., Crutzen, H.P. An inventory of ready-to-use and publicly available tools for the safety assessment of nanomaterials. NanoImpact 12, 18-28, 2018.

OECD. Test Guideline No. 202: Daphnia sp. acute immobilisation test. 2004.

OECD. Guidance document on the validation of (quantitative) structure-activity relationship [(Q)SAR] models. OECD, 2007. Available via: https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2007)2

OECD. Guidance document for the testing of dissolution and dispersion stability of nanomaterials and the use of the data for further environmental testing and assessment strategies Series on Testing and Assessment No. 318 (ed), OECD Environment Directorate, Environment, Health and Safety Division 2 rue André-Pascal 75775 Paris Cedex 16 France. 2020.

Peijnenburg W., M. Baalousha, J. Chen, Q. Chaudry, F. Von der Kammer, T. Kuhlbusch, J. Lead, C. Nickel, J. Quik, M. Renker, Z. Wang, A. Koelmans. A review of the properties and processes determining the fate of engineered nanomaterials in the aquatic environment. Crit. Rev. Environ. Sci. Technol., 45, 2084-2134, 2015.

Toropova, A.P., Toropov, A.A., Benfenati, E., Gini, G., Leszczynska, D. and Leszczynski, J. CORAL: quantitative structure–activity relationship models for estimating toxicity of organic compounds in rats. Journal of computational chemistry, 32, 2727-2733, 2011.