PATROLS
Advanced Tools for NanoSafety Testing

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PATROLS Standard Operating Procedures (SOP)

In vitro Dosimetric Model

This is a SOP used by members of PATROLS only

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1 Introduction:

DOMAIN: Other

The dynamics of deposition of Engineered Nanomaterials (ENM) in a in vitro setting can be described mathematically. The mathematical construction of the time course of deposition of ENM, the in vitro dosimetry (IVD) model, is based on a system of ordinary differential equations. The kinetics parameters of the equations are estimated using a non-linear least squares methods. The model was used to describe the kinetics of deposition of several ENM (DQ12 Quartz, CeO2, BaSO4 and TiO2) in a single and a repeated dosing experiments.

1.1 Scope and limits of the protocol

This research protocol elaborates on mathematical construction of the model, and describes the methodology behind the parametrization of the model. This SOP can be used to implement and parametrize the IVD model is intended to be used in the in vitro domain of exposure to nanomaterials. The parametrization of the IVD model is necessary to apply model to a specific ENM. Therefore, the parametrization described in this SOP requires (high quality) empirical distribution data of the nanomaterial under study. Without the emperical data, this SOP can not be fully performed. Finally, it must be noted that a IVD model parametrized to predict the kinetic distribution of a particular nanomaterial should solely be used predict the deposition of that ENM, as it possibly does not generalize well to other ENMs, without re-parametrizing the model.

1.2 *Validation state of protocol*

Level of advancement towards standardization	**Level reached** (please mark only one with "X")
Stage 1: Internal laboratory method under development	X
Stage 2: Validated internal laboratory method	
Stage 3: Interlaboratory tested method	
Stage 4: Method validated by Round Robin testing	
Standardisation plans	
Is the method considered for standardisation (OECD SPSF or similar)?	N
Has the method been submitted for standardisation (to OECD, CEN, ISO,…) in its own right or as part of another standardisation project?	N
Is the method included in an existing standard (or ongoing standardisation work)	N

2 Terms and Definitions:

Nanoscale
Length range approximately from 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from larger sizes are predominantly exhibited in this length range.

[SOURCE : ISO/TS 80004-1: 2016, definition 2.1]

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

Note 1 to entry: This generic term is inclusive of *nano-object* and *nanostructured material*.

[SOURCE: ISO/TS 80004-1: 2016, definition 2.4]

Engineered nanomaterial
Nanomaterial designed for specific purpose or function

[SOURCE: ISO/TS 80004-1: 2016, definition 2.8]

Particle
Minute piece of matter with defined physical boundaries.

Note 1 to entry: A physical boundary can also be described as an interface.

Note 2 to entry: A particle can move as a unit.

Note 3 to entry: This general particle definition applies to *nano-objects*.

[SOURCE: ISO 26824:2013, 1.1]

Substance
Single chemical element or compound, or a complex structure of compounds.

[SOURCE: ISO 10993-9:2009, definition 3.6]

3 Abbreviations:

ENM	engineered nanomaterial
IVD	*in vitro* dosimetry
ODE	ordinary differential equation

4 Principle of the Method:

IVD model is used to quantitatively simulate the deposition and distribution of ENM in a in vitro setting. IVD model describes the kinetic transport between the different in vitro compartments by rate equations, by means of coupled ordinary differential equations (ODEs). Systems of coupled ODEs are typically solved numerically using programming software.

In order to accurately describe the in vitro kinetics of ENMs, the rates with which nanomaterials are transferred from one in vitro compartment to another need to be known. Estimating values of the model parameters that describe this transfer is essential for the use of a IVD model. In this SOP (section 5.3), the estimation of IVD parameter values using a non-linear least squares method is described.

5 Description of the Method:

5.1 Apparatus and equipment used:

The IVD model simulations were run on a computer with 8 different CPU cores (each containing 8GB RAM). The IVD model was implemented in the Matlab modelling language, using a dedicated ODE solver package ODE45. The following software packages were used to run the simulations:

Name software: MATLAB (v. 2020b)

Manufacturer: Mathworks

Place of manufacture: https://www.mathworks.com/products/matlab.html

Year of manufacture: 2019

Description: A programming language for statistical computing

5.2 Quality control & acceptance criteria:

The quality of the IVD model can be validated by predicting the kinetic deposition of the nanomaterial under study on an independent test dataset. Such a test set is a set of measured nanomaterial concentrations in a in vitro model acquired after an initial dosing scenario that is independent from the initial dataset used to parameterize the IVD model. The predicted nanomaterial kinetics were subsequently compared to emprically measured nanomaterial deposition of the test set. If the predictions correspond well with the measured concentrations, then it can be concluded that the IVD model is likely to generalize well to other in vitro exposure scenario's.

5.3 Procedure:

General model:

In FP7 SUN a kinetics model describing the dose-response in simple *in vitro* models. The model describes the distribution of the deposited dose into the *in vitro* cell population. IOM will further adapt this model for the more sophisticated *in vitro* models developed in PATROLS. In T1.4, *in vitro* experiments on A549 cells to monitor material uptake, intracellular fate and translocation across the cellular membrane were performed. The ENM used in the experiments were: TiO2 (NM105), DQ12 (NM200), ZnO (NM110), BaSO4 (NM220) and CeO2 (NM212). Two sets of experiments were conducted. The dosing regimen is summarized in Fig 1.

Fig.1: *The dosing regimen in the one exposure and repeated exposure experiments*

The fraction of ENM were measured in the different compartments described in Fig 2.

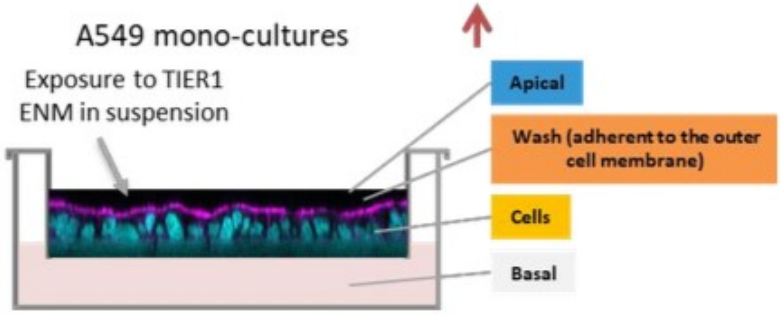

Fig 2 *The different compartments where fraction of deposited dose were measured: (1) Apical, (2) Wash, (3) Cells and (4) Basal.*

Materials and Methods

The model is adapted to simulate the data obtained from T1.4. The model consists of four compartments Apical (A), Wash (W), Cells (C) and Basal (B). The kinetics of ENM translocation in each compartment are described by a series of differential equations:

$dA/dt = -k_0Adt$
$dW/dt = k_0Adt – k_1Wdt$ (2) $dC/dt = k_1Wdt - k_2Cdt$
$dB/dt = k_2Cdt$

The model is summarised in Fig 3.

The parameters k_i (i=0,1,2) were estimated using non-linear least squares using the dataset from the first experiment for each ENM. Mathematically, the k_i were chosen to minimise the sum of squares of the model simulated values, at time=24, for A, W, etc...and their experimental values at the same time.

The model is written in MATLAB and the numerical routine '*fmincon*' of the MATLAB statistical toolbox was used for minimisation in this exercise.

1. The model is calibrated using the dataset of experiment 1 in which a dose of 100 µg/mL is applied at time zero and measurements were made at t=24hrs to obtain the parameters.

2. The model is calibrated again using the second experiment for the first 24hr (when a dose of 25 µg/mL is administered at t=0) and the parameters estimated again because the parameters are likely to be dependent on the initial mass dose.
3. The model is then used to simulate the outcomes at 48, 72 and 80 hr for model validation.

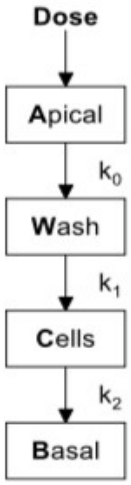

Fig 3. *The IVD model and the parameters ks.*

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6 Data Analysis and Reporting of Data:

After estimating the optimal values of the parameters (i.e., parameterizing) in the IVD model, the quality of the model was validated on an independent test set. In this context, the IVD model is used to predicted nanomaterial deposition in a in vitro dosing scenario that is different from that used to parameterize the model. The model predictions are subsequently compared to the empirical measurements of the nanomaterial concentrations in a in vitro setting. To allow for qualitative visual comparison, the predicted nanomaterial concentrations were plotted over time and compared to nanomaterial concentrations empirically measured in vitro studies.

Concerning the parametrization of the model, a non-linear least squares method was developed. The estimated parameters were the parameter values which minimized the sum of squares between the model predictions and the observed values.

7 References

Matlab (2020b). **https://www.mathworks.com/products/matlab.html**