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Development of PBPK models

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4		

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Table of Content

1 DESCRIPTION OF TASK 4

2 DESCRIPTION OF WORK & MAIN ACHIEVEMENTS 4

2.1 SUMMARY 4

2.2 BACKGROUND OF THE TASK 5

2.3 DESCRIPTION OF THE WORK CARRIED OUT 5

2.3.1 Study objectives 5

2.3.2 Data collection 5

2.3.3 Development of PBPK model 6

2.4 RESULTS 8

2.4.1 PBPK model for intravenous exposure 8

2.4.2 PBPK model for inhalation exposure 10

2.5 EVALUATION AND CONCLUSIONS 11

2.6 DATA MANAGEMENT 11

3 DEVIATIONS FROM THE WORK PLAN 11

4 PERFORMANCE OF THE PARTNERS ..... ERROR! BOOKMARK NOT DEFINED.

5 REFERENCES / SELECTED SOURCES OF INFORMATION (OPTIONAL) 11

6 LIST OF ABBREVIATIONS (OPTIONAL) 12

1 Description of task

The aim of the project is to develop nanospecific physiologically based pharmacokinetic (PBPK) models that describe the uptake and fate of nanoparticles in the body. In similarity with conventional PBPK models, the model will combine a biologically relevant model structure, agent-independent parameters e.g. pulmonary ventilation, organ blood flows, organ volumes, and agent-dependent parameters e.g. alveolar ventilation, phagocytosis, transport across cellular membranes, and protein corona formation.

PBPK modelling requires access to in vivo biodistribution data covering multiple organs and sampling times. Along with the generation of such data within the NANoREG project, conceptual models are developed in parallel but based on published data until generated data are available. This work contributes with information for occupational exposure and consumer health.

2 Description of work & main achievements

2.1 Summary

A conceptual nanospecific physiologically-based pharmacokinetic (PBPK) model for intravenous administration to rats was developed and applied on different types of inert nanoparticles using experimental data from recently published scientific publications. The model represents systemic distribution and serves as a foundation for expansion to other species and to other exposure routes (inhalation, dermal, oral). The results suggest that the model structure can be used to describe the biokinetics of different types of inert nanoparticles despite large differences in properties and exposure conditions. The model is the first to include separate compartments for phagocytic cells and saturable phagocytosis. The simulations show that (1) phagocytosis need to be incorporated in nano-PBPK models, (2) the dose exerts a profound impact on the biokinetics, and (3) additional information concerning the permeability of nanoparticles in different organs is required.

The PBPK model was expanded to include inhalation exposure using data from a short term inhalation exposure to aged and pristine nanoceria in rats. The model includes mucociliary clearance, olfactory uptake, phagocytosis, and entry into the systemic circulation by alveolar wall penetration. The PBPK model described the biodistribution well and again suggested phagocytosis to be very important.

Despite some major achievements, these nano-PBPK models are still in their infancy and cannot yet be readily used in the regulatory arena. On the other hand, PBPK modeling provides valuable information about uptake and distribution but need to be further refined before they can be successfully used as regulatory tools. To further develop and improve the nano-PBPK models, more informative experimental biodistribution data are needed, including:

1) extensive characterization
2) monitoring of several organs at several time-points
3) frequent sampling immediately after dosing
4) long follow-up post-dosing
5) account for mass balance (total recovery)
6) detailed description of the analytical procedures

Many of these requirements are fulfilled in different NANoREG projects, however, the results from these experiments have only now become available. We therefore had to rely on previously published experimental data.

2.2 Background of the task

Toxicity depends on the target dose. To predict target dose information about kinetics is essential, as reflected in regulatory question 6, 7, 8. PBPK models can provide valuable input in this respect. PBPK models may be used in several ways:

- To describe, understand and predict how nanoparticles are taken up, distributed, degraded and excreted from the body
- For species extrapolations (e.g. from *in vitro* and animals to humans)
- For route extrapolations (e.g. between intravenous, oral, dermal and inhalation)
- To predict the biokinetics and target doses for new exposure scenarios
- In biomarker development (correlation between dose/exposure and biomarker)
- To estimate population variability in target doses and biomarkers
- To generate and test hypotheses regarding biokinetic mechanisms

PBPK modelling reduces the need for in vivo experiments s do not require additional in vivo tests to make new predictions when exposure conditions and species are changed. Consequently, time and resources can be saved.

2.3 Description of the work carried out

2.3.1 Study objectives

The objective was to develop nanospecific PBPK models that can be used to describe and predict the biokinetics of nanoparticles. The design and application of the model should provide information about rate limiting and critical processes for uptake, distribution, metabolism and excretion of nanoparticles.

2.3.2 Data collection

At the start of NANoREG project no data on NANoREG core materials suitable for development of nano-PBPK models had yet been generated. Therefore, as a starting point, data on polyethylene glycol coated polyacrylamide nanoparticles injected intravenously in rats were used to construct an initial model. This data set was very suitable for modelling as it contained nanoparticle concentrations in multiple organs over time, and also reported the total recovery. Later on, data on other inert nanoparticles, suitable for modelling, was collected from the literature. Totally only three additional data sets were found covering titanium dioxide, gold and uncoated polyacrylamide nanoparticles, Table 1. An effort was also made to use the model to compare systemic biodistribution of nanoceria after inhalation, instillation and inhalation exposure.

Table 1. Summary characteristics of the studies included in development of the nanospecific PBPK model.

Material	***PAA***	***PAA***	***Gold***	***TiO_2***
Trade name	Synthesized	Synthesized	Synthesized	Degussa P25
Coating	PEG	No	CTAB	No
Shape	Sphere	Sphere	Rod	Sphere

Size (nm)	D:31	D:31	L:56 D:13	D:63
Size method	DLS	DLS	TEM	DLS
Dose (mg/kg)	28	45	0.56	0.95
Sampling times (h)	0.08, 0.17, 0.5, 1, 4, 8, 24, 48, 72, 96, 120	0.08, 0.17, 0.33, 0.67, 1, 2, 4, 8, 24, 72, 96, 120	0.5, 1, 4, 16, 24, 72, 168, 336, 672	6, 24, 72, 168, 720
Rat strain	Crl CD®(SD)IGS BR	Crl CD®(SD)IGS BR	Sprague - Dawley	F344/DuCrlCrlj
Number of animals	2-3	2-3	3	5
Organs collected	Bl, Li, Sp, Lu, Ki, He, Br, Lymp, BM, Carcass	Bl, Li, Sp, Lu, Ki, He, Br, Lymp, BM, Carcass	Bl, Li, Sp, Lu, Ki, He, Br, Bo, Mu	Bl, Li, Sp, Lu, Ki, He, Br, Lymp
Excretion	U+F	U+F	U+F	U+F
Analytical method	C14	C14	ICP-MS	ICP-SFMS
Reference	Wenger et al 2011	Wenger et al 2011	Wang et al 2010	Shinohara et al 2010

Abbreviations: PAA - polyacrylamide, PEG - polyethylene glycol, CTAB - Cetyltrimethylammonium bromide, TiO2 - titanium dioxide, Bl - Blood, Li - Liver, Sp - Spleen, Lu - Lung, Ki - Kidney, He - Heart, Br - Brain, Lymp - Lymph nodes, BM - Bone Marrow, Bo - Bone, Mu - Muscle, U – Urine, F – Feces, DLS – Dynamic Light Scattering, TEM – transmission electron microscopy, C14- carbon-14 radioactivity, ICP – Inductively Coupled Plasma, MS – Mass Spectroscopy, SFMS - Sector Field Mass Spectroscopy, D – Diameter, L – Length.

Long term inhalation studies on nanoceria are tasks within NANoREG, but data on biodistribution became available very late in the project, which hindered the availability for modelling. To prepare modelling for that data, we expanded the model to inhalation exposure using another data set, available at time of modelling. The data set covered short term inhalation studies of aged and pristine nanoceria. The modelled nanoceria was generated via a combustion method. Aging was carried out in urban air simulated conditions combined with UV irradiation. TEM analysis indicated that primary particle size was 2-3 nm but because of agglomeration the inhaled size increased and became bimodal 25 and 90 nm, according to SPMS single particle mass spectrometry) measurements. Spraque Dawley rats were exposed for 4 h in a nose-only exposure chamber. Concentration ranged between 172 to 1240 µg/m3. After exposure, three rats each were sacrificed after 15 min, 24 h, and 7 days (n=3 per time point and experiment) and blood, lungs, liver, kidneys, heart, brain, olfactory bulb, and spleen were harvested. Feces and urine were collected for first 24 h post exposure. Cerium levels in organs and in urine and feces were analyzed ICP-MS.

2.3.3 Development of PBPK model

The methodology to development a nanospecific PBPK model followed the principles of conventional PBPK models and can be subdivided into the following parts

1. Literature review and evaluation

2. Identification of critical processes and rate limiting factors in absorption, distribution and clearance of nanoparticles
3. Develop a generic physiological model describing mammal (in this case rats)
4. Mathematical description of the model as mass-balance equations
5. Identification of parameter values for the model (values taken from the literature and/or by fitting the model to experimental data)
6. Comparison between experimental data and in vivo data
7. Evaluation with independent data

A conceptual model was constructed based on findings from literature review and organs analysed in study on PAA-PEG injected intravenously into rats. In the next step the model was refined and optimized to data sets on PAA, gold and titanium dioxide. The model consists of 10 compartments; arterial blood, venous blood, liver, spleen, lung, kidney, heart, brain, bone marrow and carcass. Each compartment is divided into three sub compartments: capillary blood, tissue and phagocytic cells.

The exchange of nanoparticles between blood and tissue in each organ is described by flow- and diffusion-limited processes. Diffusion between blood and tissue is controlled by a permeability coefficients, which limits the effective blood flow. The uptake by phagocytic cells designed to be saturable.

Next the model was expanded to include inhalation exposure by including deposition in the respiratory system and transfer to the gastrointestinal tract. Following inhalation, the nanoparticles are deposited into three regions of the respiratory system: the upper airway, the tracheobronchial region, and the pulmonary region. From the upper airway deposited nanoparticles can migrate either to the brain via the olfactory system, or to the gastrointestinal tract by being swallowed. Nanoparticles deposited in the tracheobronchial region are transferred to the pharynx in the upper airway by mucociliary clearance, then swallowed to the gastrointestinal tract. In the gastrointestinal tract nanoparticles can be taken up into the systemic circulation or transported distally to feces. From the lumen of the lungs, nanoparticles can translocate into interstitium and further on to the systemic circulation system. Via the systemic circulation nanoparticles are distributed to organs. Nanoparticles deposited in in the pulmonary region can be engulfed by alveolar macrophages followed by migration to the upper airway by mucociliary transportation.

Implementation and parameterization of the model was carried out in Berkeley Madonna version 8.3.18 (Berkeley, CA) and acslX_Libero version 3.0.2.1. The model includes parameters, which are physiologically based or nanoparticle dependent. Physiolgoical parameters are typically body and organ weights weight and blood flows. These parameters were taken from the literature or from the experiments. Nanoparticle dependent parameter were optimized to provide the best fit between predictions and experimental observations. Nanoparticle dependent parameters in the models include; uptake and release rate to phagocytic cells, uptake capacity of phagocytic cells in tissues, partition between blood and tissue, and permeability.

Evaluation of the performance of the models were carried out by visual inspection followed by comparing log likelihood values (acslX Libero™), R2 values (linear regression analyses of log values), and PBPK indices (using log values).

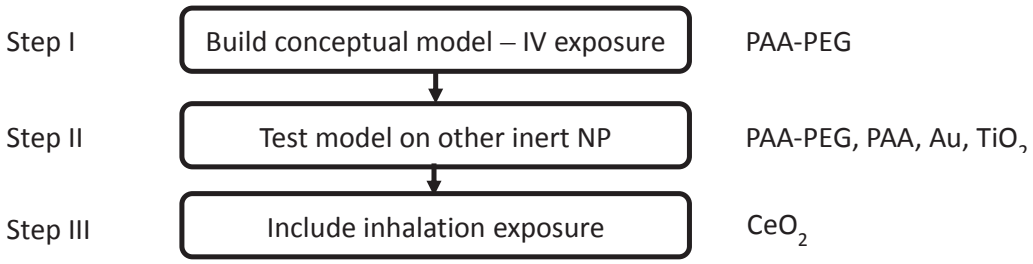

Figure 1. Steps in the model development.

2.4 Results

2.4.1 PBPK model for intravenous exposure

In this delivery the development of a conceptual model for systemic distribution is described.

A nanospecific PBPK model for systemic distribution following intravenous administration was developed. The model describes the biokinetics of four different types of inert nanoparticles despite large differences in properties and exposure conditions.

PBPK models are built on knowledge from in vitro and in vivo studies. A key process for deposition in tissue and organs for nanoparticles is the internalization of nanoparticles by phagocytic cells in the mononuclear phagocyte system (MPS). When nanoparticles are injected to the blood, the majority are captured by the MPS in phagocytic cells, especially in the liver and spleen. The kinetics of the phagocytosis process depends on the properties of the nanoparticles. Several properties have been found to change the biokinetic behaviour as size, charge, hydrophilicity, surface structure, shape, corona formation and agglomeration status. To address these issues we assumed the portioning between blood and tissue interstitium and the affinity and capacity of phagocytizing cells to be nanoparticle dependent. Meanwhile, the number of phagocytizing cells was assumed to be nanoparticle independent but was allowed to vary between tissues.

To control if models can predict the biokinetic behaviour of nanoparticles, the model has to be evaluated against in vivo data from biodistribution studies. In order to find biodistribution data suitable for modelling we did a literature search on gold, silver titanium dioxide, silica and polymeric nanoparticles. These types of nanoparticles represent a wide range of physical properties and fields of application. The search was limited to single dosed intravenous injected nanoparticles in rats. We found several studies but the majority of published studies were not useful for PBPK modelling, see Table 2. The following limitations were identified: (1) incomplete NP and dose characterization, (2) short follow-up post-dosing, (3) few samples per tissue, (4) few tissues/organs studied, and (5) failure to account for the mass balance, and (6) lack of confirmation of NP integrity in the tissues. These shortcomings make time course descriptions, half time calculations and estimates of bioaccumulation uncertain. Overall, our review indicates that it is difficult to draw general conclusions about nanoparticle biodistribution. With the limited data at hand, it seems that no individual factor such as size, coating, shape, charge, chemical composition or agglomerations status can explain the biodistribution. In conclusion, the ADME of NP is complex.

Table 2. Summary of elements that are reported to influence biodistribution and hence PBPK modelling of nanoparticles based on literature review from 2014.

	Number of studies						
Elements reported	**Ag**	**Au**	**Polymer**	**Silica**	**TiO2**	**Total**	**%of Total**
Size ≤ 100 nm	5	55	69	19	3	151	**62%**
No coating	2	17	45	14	3	81	**33%**
Pegylation	3	22	73	10	1	109	**45%**
Other coating	0	23	22	9	0	54	**22%**
Surface area	2	11	28	2	2	45	**18%**
Number of particles	0	25	19	2	0	46	**19%**
Z –Potential	2	36	81	8	4	131	**54%**
Time course > 1week	2	8	9	12	4	35	**14%**
Time course ≥ 3 time points	4	26	33	25	4	91	**37%**
More than 4 organs	5	60	65	28	4	162	**66%**
Levels in blood	5	58	128	21	4	216	**89%**
Levels in liver	5	62	127	30	4	228	**93%**
Levels in spleen	3	58	139	30	4	234	**96%**
Levels in lung	5	58	44	28	4	139	**57%**
Levels in kidney	5	60	23	27	4	119	**49%**
Excretion	0	21	8	6	2	55	**23%**
Total number of data sets	**5**	**62**	**140**	**33**	**4**	**244**	**100%**

Based on a biodistribution study in rats on polyethylene glycol coated polyacryl amide (PAA-PEG), an intravenous single dose PBPK model was developed and published. The PAA-PEG nanoparticle was selected because the study contained detailed experimental data (time courses of NP mass in different tissues). The model consists of 10 compartments and addresses the mononuclear phagocyte system. Including the phagocytic process is new compare to previous models. Each compartment has three sub-compartments: capillary blood, tissue and phagocytic cells.

The model explains 97% of the observed variation in nanoparticles amount across organs. According to the model, phagocytizing cells quickly capture nanoparticles until their saturation and thereby constitute a major reservoir in richly perfused organs (spleen, liver, bone marrow, lungs, heart, kidney), storing 83% of the nanoparticles found in these organs 120 hours after injection. Figure 2 describes the deposition in of nanoparticles in the liver sub-compartments where the majority is found in phagocytic cells. Key determinants of the nanoparticles biodistribution are the uptake capacities of phagocytizing cells in organs, the partitioning between tissue and blood, and the permeability between capillary blood and tissues.

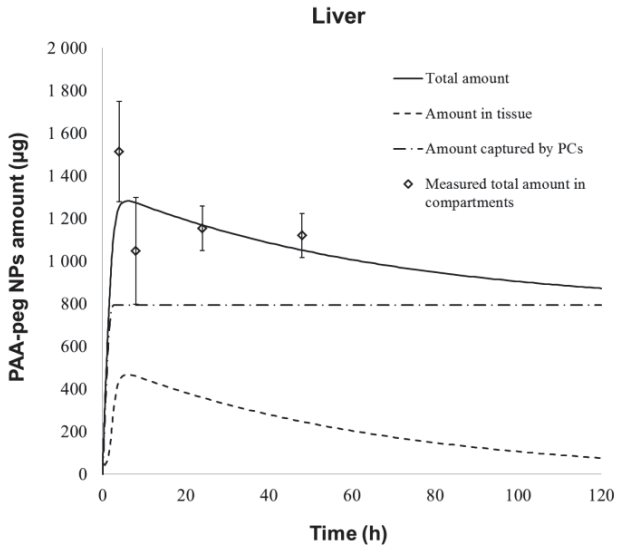

Figure 2. Time courses of PAA-peg nanoparticles in liver sub-compartments predicted by the PBPK model versus measured data. Error bars show the standard deviation of measured data. PCs - phagocytizing cells.

To evaluate if the PAA-PEG model can be used as a general model we tested the model on three additional types of nanoparticles; PAA, Gold and Titanium Dioxide. The model adequately describes the biokinetic behaviour of all four NP types only by adjusting nanoparticle related parameters (R^2 on the log scale ranging from 0.88 to 0.96). The fitted parameters that varied most were the clearance to urine and faeces, the blood:tissue permeability coefficients and the uptake rate and capacity of phagocytic cells. Additional data on NP properties such as corona formation and physiological parameters, such as number of phagocytic cells in different tissues and their capacity and turnover, are required to further improve the model.

2.4.2 PBPK model for inhalation exposure

In this delivery the development of a nanospecific PBPK model for inhalation exposure is described.

The model in 4.2.1 was expanded to include inhalation and describes the biodistribution of short term inhalation of nanoceria in rats well.

The expanded PBPK model describes the biodistribution of inhaled nanoceria well and is able to reproduce the different experimentally observed trends (R^2 on the log scale ranging from 0.68 to 0.95).

Despite large differences in haled dose, the amount of smaller nanoceria particles deposited in the pulmonary region varied less than 31% and the amount of CeO2 recovered in all the extrapulmonary organs was quite similar (within 65 % of each other). Interestingly, the deposition fractions in the tracheobronchial and pulmonary regions calculated by the expanded PBPK model were lower than that calculated from the Multiple-Path Particle Dosimetry Model (MPPD v2.11) while the deposition fraction in the upper airway region was higher. In fact the majority of nanoceria was recovered in feces followed by the lung. In total less than 4% was detected in extrapulmonary organs.

The model predictions were sensitive to changes in model parameters as the fraction of inhaled nanoparticles deposited in the upper airway and in the pulmonary region, the feces clearance

rate from the GI tract, uptake rate from GI tract, partition between blood and tissue and permeability coefficient connected to lung, kidney, heart and carcass.

2.5 Evaluation and conclusions

To our knowledge, the conceptual model developed within this task is the first one to include a separate compartment for saturable phagocytic cells. This structure has later been adapted and modified in other published models, which supports its importance in nanospecific PBPK models.

In agreement with the results from experimental biodistribution studies, our modelling exercises demonstrate that kinetics depends on both nanoparticles properties and exposure conditions.

Despite some major achievements, these nano-PBPK models are still in their infancy and cannot yet be readily used in the regulatory arena. On the other hand, PBPK modeling provides valuable information about uptake and distribution but need to be further refined before they can be successfully used as regulatory tools. To further develop and improve the nano-PBPK models, more informative experimental biodistribution data are needed, including:

1) extensive characterization
2) monitoring of several organs at several time-points
3) frequent sampling immediately after dosing
4) long follow-up post-dosing
5) account for mass balance (total recovery)
6) detailed description of the analytical procedures

2.6 Data management

The ISA-TAB-Nano template was delivered in time and according to the project office implemented in the reporting tool. However, the NANoREG report tool requires NANoREG core material to make registration possible. Our deliveries are based on data from the literature and not NANoREG core materials. We were therefore unable to report our results in the tool.

3 Deviations from the work plan

Nothing to report

4 References / Selected sources of information (optional)

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5 List of abbreviations (optional)