HUMAN EQUIVALENT CONCENTRATION AND KINETIC MODELLING OF AEROSOLS IN THE LOWER RESPIRATORY TRACT

RESEARCH PROJECT F2437: Derivation of occupational exposure limits for airborne chemicals – Comparison of methods and protection levels

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Content

Extended Summary ........................................................................................................... 5
Abbreviations .................................................................................................................. 10

1 Introduction ............................................................................................................... 13

2 Definitions and Demarcation .................................................................................... 15
  2.1 Human Equivalent Concentration (HEC) .............................................................. 15
  2.2 Particle properties ................................................................................................. 16
  2.3 Effects in the upper respiratory tract ................................................................. 17
  2.4 Nanoparticles ....................................................................................................... 17

3 Weighted Breathing Volume: AgVT/AgVH .............................................................. 18
  3.1 Breathing volume comparisons ............................................................................ 18
  3.2 Current quantitative approach in Germany ......................................................... 18
  3.3 New data ................................................................................................................ 19
  3.4 AgVT / AgVH from mice data ................................................................................. 21
  3.5 Conclusions .......................................................................................................... 24

4 Deposition fraction (DFT/DFH) .............................................................................. 26
  4.1 Deposition fraction – overview ............................................................................ 26
  4.2 Alternative models for deposition modelling ....................................................... 27
  4.3 MPPD deposition modelling ................................................................................ 28
    4.3.1 MPPD Version 2.11 vs. Version 3.04 ............................................................ 28
    4.3.2 MPPD (Version 3.04) application ................................................................. 28
    4.3.3 Quantitative changes (MPPD 3.04 vs. MPPD 2.11) .................................... 29
  4.4 Deposition and region of the lower respiratory tract ............................................ 32
  4.5 Inhomogeneous deposition .................................................................................. 33
  4.6 Deposition and solubility ..................................................................................... 33
  4.7 Deposition and density ......................................................................................... 34
  4.8 Deposition and particle size ................................................................................ 36
  4.9 Inhalability adjustment, applying MPPD ............................................................. 41
  4.10 Deposition variability ......................................................................................... 41
  4.11 MPPD deposition for mice ................................................................................ 43
4.12 Summary and conclusions on deposition ............................................................. 44

5 Normalising Factor (NFH / NFt) and Dose Metrics .............................................. 46
  5.1 Normalisation and dose metrics – overview ..................................................... 46
  5.2 The German PSLT-approach and dose metrics/ normalisation ....................... 47
  5.3 Alternatives in dose metrics ............................................................................ 48
  5.4 Alternatives in normalisation ......................................................................... 49
  5.5 Influencing factors: mode of action and solubility ......................................... 52
  5.6 Mice specific normalisation ......................................................................... 53
  5.7 Summary and conclusions on normalisation and dose metrics ....................... 53

6 Retention and Elimination (ELRH / ELRT) ..................................................... 55
  6.1 Retention and elimination overview .............................................................. 55
  6.2 Clearance mechanisms and species differences ........................................... 56
  6.3 Current handling of elimination .................................................................. 56
  6.4 Translocation to the interstitium and consequences for interspecies elimination rates ................................................................. 57
  6.5 Clearance impairments at high exposure concentrations ............................. 59
  6.6 Elimination of soluble particles ................................................................. 60
  6.7 Variation in clearance due to respiratory illnesses, individual differences and local inhomogeneity ............................................................... 62
  6.8 Interspecies differences in elimination rate from mice experimental studies .... 62
  6.9 Summary and conclusions on retention and elimination ............................ 63

7 Aggregated HEC-Calculation .......................................................................... 65
  7.1 Aggregated HEC-calculation – overview ....................................................... 65
  7.2 HEC – no isolated precursor step ............................................................... 65
  7.3 Partial HEC, if only selected data are available? .......................................... 66
  7.4 Suggested “aggregate 3 ratios approach” ..................................................... 67
  7.5 Aggregated HEC calculation based on experimental data for mice .............. 70
  7.6 Translocation to the interstitium: suggested separate sub-factor in HEC default calculations ............................................................. 71
  7.7 Range constraints for the HEC-approach ...................................................... 72
  7.8 Some examples for HEC ............................................................................ 73
  7.8.1 HEC - calculation from rat data for PSLT substances: titanium dioxide .... 73
7.8.2 HEC – calculation from rat data for water-soluble particles: cobalt sulfate
........................................................................................................................................ 75

7.8.3 HEC – calculation from rat data for particles with lysosomal solubility: cobalt metal
....................................................................................................................................... 77

7.8.4 HEC – calculation from mice data for particles with lysosomal solubility: cobalt metal
........................................................................................................................................ 80

7.8.5 Conclusions from the examples ................................................................................. 82

7.9 Summary of uncertainties in the HEC approach .......................................................... 83

8 References .......................................................................................................................... 85
Extended Summary

The “Human Equivalent Concentration” (HEC) approach is a procedure to extrapolate an exposure concentration from an experimental animal study to an equivalent human concentration for a chronic workplace inhalation exposure scenario. Within this report we discuss HEC calculations for solid particles in the lower respiratory tract. Recent developments of the HEC approach (scientific update; new calculation procedures; improvements; uncertainties) are described and compared to earlier versions. This analysis can be used by regulatory bodies to establish guidance on how to apply the HEC approach in regulatory procedures aiming at deriving occupational exposure limits (OEL) for particles affecting the lower respiratory tract.

A four step procedure

Exposure concentrations of particles in experimental animal studies are not regarded as equivalent to workplace concentrations due to several reasons: i) the intake into the lower respiratory tract depends on breathing patterns (nose or mouth breathing); also, breathing frequency and breathing volume differ significantly between rodents and humans, ii) the morphology of the human respiratory tract is different from that of rodents, which consequently leads to differences in deposition of particles in the lower respiratory tract including the lung; for example, a much higher fraction of respirable particles with a Mass Median Aerodynamic Diameter (MMAD) of more than 2 µm is deposited in the deep human lung compared to the rat lung; iii) once the particles reached the lung, the respective contact sites in the two species (usually rats and humans are compared) are highly different with regard to volume or surface area at the contact sites; defence or adverse responses or other biological reactions in the local lung environment will be initiated and in consequence interspecies differences of, e.g., the alveolar surface areas or the macrophage capacity may subsequently lead to different responses; iv) finally, translocation within the lung and elimination of particles from the lung have been observed to be highly different between many animal species and humans.

HEC aims at correcting the external concentration for differences between animals and humans with regard to the retained dose of particles in the lung. Consequently, a multi-step procedure is taken: four interspecies ratios are calculated and then multiplied: (1) the weighted daily breathing volume for animals vs. humans, (2) a deposition fraction ratio, (3) a normalising factor ratio, (4) an elimination rate ratio. HEC is derived by multiplying the exposure concentration from the animal study (c_T) by these four ratios according to the following formula:

\[ HEC = c_T \times \frac{AgV_T}{AgV_H} \times \frac{NF_H}{NF_T} \times \frac{ELR_H}{ELR_T} \times \frac{DF_T}{DF_H} \]

where

\( T \) indicates animal data (“animal” in German language: “Tier”), \( H \) human data, \( c_T \) is the exposure concentration from the animal study, for which we want to know the human equivalent,
AgV is the weighted breathing volume per day (German: “gewichtetes Atemvolumen”)
NF is a normalising factor,
ELR is the elimination rate, and
DF is the deposition fraction.

The most frequent starting points for interspecies extrapolation are rat studies. However, the use of mice studies is also briefly addressed.

The ratio for the weighted daily breathing volume \((AgV_T/AgV_H)\)

In earlier versions of the HEC approach, the ratio for the weighted daily breathing volume has been a fixed value with data from one rat strain and from working persons. New data permit to consider more specific input from several rat strains and with different animal body weights.

For experimental data on breathing volumes of rats, a high variability is documented; various existing allometric regression formulae result in different breathing volumes at identical body weights. A currently suggested default value of 0.008 for this ratio apparently is a conservative approach. This value of 0.008 means that the weighted daily breathing volume in rats of 0.055 m³/day is assumed and compared to a weighted daily breathing volume of workers for chronic exposure of 6.57 m³/day \((0.055/6.57 \approx 0.008)\). Therefore, calculation procedures for various values are discussed considering the impact of strain and body weight on breathing volume of the experimental animals in the assessed study. Uncertainties of respective calculations are addressed accordingly.

The deposition fraction ratio \((DF_T/DF_H)\)

The deposition fraction is calculated by dosimetric modelling in both species (e.g., rats and humans). This modelling includes mechanistic considerations and fluid dynamics with respect to sedimentation, impaction and diffusion of particles, with special consideration of the particle sizes and of the anatomy of the respiratory tract with different air flow characteristics in the upper, tracheobronchial and the pulmonary region.

Deposition within the HEC approach in this project is calculated by modelling with the “Multiple Pathway Deposition Model” (MPPD), which is freely available as updated version 3.04. Major changes compared to the former version (version 2.11) are described and the quantitative outcome is discussed for calculations of deposition for varying particle densities or particle sizes. Major areas of uncertainties are (1) inhomogeneity of deposition with potential “hot spots”, which are not covered in subsequent HEC calculations, but might contribute to adverse effects and for which species differences are to be acknowledged and (2) hygroscopic growth of water soluble particles, as this growth in particle size is not covered by MPPD calculations, but may significantly alter deposition patterns. Deposition in the lung is significantly influenced not only by particle size but also by particle density. In interspecies comparisons, it is relevant to know whether the relative fractional deposition ratio
(rodents/humans) changes depending on size and/or density within the applicability range for default calculations.

The deposition fraction ratio as output from MPPD is a single ratio. However, in reality for both species (rodents and humans) there may be significant variability in the respective deposition fraction. Typical values for the deposition fraction ratio may be in the range of 0.2 to more than 1; this implies that the deposition fraction is frequently smaller in experimental animals than in humans, depending on the particle size in the experimental study. However, the term “more than 1” includes the possibility that a higher fraction of particles is deposited in the rodent lung than in the human lung.

From the limited data available for validation of the HEC calculations based on mice data and from an uncertainty analysis we conclude that interspecies particle deposition estimates based on mice data are associated with substantial uncertainty.

The normalising factor ratio (NFh/NFr)

Exposure needs to be quantified as a dose (measured in appropriate dose metrics) and needs to be related to a meaningful reference unit in the target organ (the lung). These steps are accomplished by assigning dose metrics to the deposited particles and by normalisation. Normalisation describes the reference unit for the deposited dose, for example, the alveolar lung surface area or the volume of the alveolar macrophages.

There is considerable variability in the data provided for either normalisation, leading to relevant uncertainty for this normalisation factor ratio. However, the most serious problem is to select the appropriate reference for normalisation. Choice of the appropriate reference might depend on the mode of action for the adverse lung effects, which is frequently insufficiently known. Further, if the average deposition in the respiratory tract is not determining the effect, but instead local deposition at hot spots is critical, this should be addressed by refined normalisation units.

Specifically, the influence of particle solubility is insufficiently correlated to the mode of action and to critical lung tissues. With solubility we refer to solubility in physiological lung fluids and not primarily to solubility in water. Solubility of the particle may greatly influence the mode of action in the respiratory tract (with respect to, e.g. primary target tissue, intracellular uptake, binding to proteins).

For poorly soluble and low toxicity particles (PSLT particles) the alveolar macrophage volume is frequently suggested for normalisation and the dose metrics used is corrected for particle density. However, many particles cannot be clearly identified as PSLT particles, and their toxicity mechanism is often unknown, although knowledge of the chemical reactivity from either the surface of the particle or the solubilized particle is highly important for adequate HEC calculations.

The normalisation factor ratio is usually a quite large value as the much larger reference term for humans is divided by the respective smaller term in the experimental animal. For example, the alveolar surface area in humans may be estimated to be
about 1,020,000 cm²; the alveolar surface area in rats is said to be 4,000 cm². This results in a ratio of normalisation factors of about 250. Note, however, that this quantification is just one of many. If the total alveolar macrophage volume is used instead, most calculated normalisation factor ratios are even larger.

The elimination rate ratio (ELR_H/ELR_T)

The potential to exert adverse health effects in the lower respiratory tract will be greatly influenced by the residence time of the particles in critical regions of the lower respiratory tract. The retention of particles in the lung is directly correlated with the respective elimination kinetics. Therefore, the fourth step of the HEC calculation is the quantification of species differences in elimination rate.

Species differences in elimination rates were formerly mostly attributed to differences in mucociliary clearance, for which different half-lives of particles in the lung of rats or humans were observed. However, species differences in elimination from the lung may also result, e.g., from translocation to the interstitium or from different retention patterns due to binding of particles to biomolecules. If a compound is retained in the lung, but is quiescent due to binding to biomolecules, i.e. not biologically active, during certain periods of time, consideration of the retained dose would be misleading. Furthermore, the assumed first order kinetics may not always be justified, and the assumption of a multi-phase elimination process be more adequate.

PSLT particles are mainly eliminated via the mucociliary escalator. For this clearance mechanism species differences are well-known. However, species differences are less evident for other clearance mechanisms (e.g. translocation to the interstitium) and it is often assumed that there are no species differences for readily soluble particles or poorly soluble particles cleared partly by other mechanisms than the mucociliary escalator. However, this is regarded to be an oversimplification.

The elimination rate ratio for PSLT particles is usually reported to be about 0.15, which acknowledges the much longer retention of particles in the human lung compared to the rat lung. Again, there is considerable variability in this ratio. This quantification does not take into account species differences in translocation to the interstitium: in some cases, the particle fraction in the interstitium should not be regarded as eliminated from the lung but may contribute to adverse effects. The ratio of 0.15 might also not be applicable to soluble particles, but to date data are insufficient for a sound quantification.

Conclusions

With the HEC procedure the starting point is adapted: HEC aims at correcting the external concentration for differences between animals and humans with regard to the retained dose of particles in the lung it. However, because of the many uncertainties of the HEC approach (as shown in example calculations) we conclude that an improved starting point will not be easily established. Considering these uncertainties, the external exposure concentration in the animal study may be used as human equivalent concentration (HEC/concentration in animal study = 1). In a more conservative
approach, a pragmatically derived assessment factor may possibly better reflect the overall uncertainties compared to highly uncertain, but scientifically refined quantitative ratios. Suggestions for such pragmatically derived assessment factors are given in this report. The consequences of either approach are presented and briefly discussed.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AGS</td>
<td>Ausschuss für Gefahrstoffe (Committee on Hazardous Substances in Germany)</td>
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<tr>
<td>AGW</td>
<td>Arbeitsplatzgrenzwert (identical: German OEL)</td>
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<tr>
<td>AgV</td>
<td>Weighted breathing volume / d (gewichtetes Atemvolumen) / Tag</td>
</tr>
<tr>
<td>AgV_H</td>
<td>Weighted breathing volume for humans (H = humans)</td>
</tr>
<tr>
<td>AgV_T</td>
<td>Weighted breathing volume for animals (T = animals)</td>
</tr>
<tr>
<td>AM</td>
<td>Aleveolar macrophages</td>
</tr>
<tr>
<td>BAuA</td>
<td>Bundesanstalt für Arbeitsschutz und Arbeitsmedizin</td>
</tr>
<tr>
<td>bpm</td>
<td>Breaths per minute</td>
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<tr>
<td>BW</td>
<td>Body weight</td>
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<tr>
<td>c_T</td>
<td>Concentration in animal study (T = animals)</td>
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<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>DEF</td>
<td>Deposition enhancement factor</td>
</tr>
<tr>
<td>DF</td>
<td>Deposition fraction</td>
</tr>
<tr>
<td>DFG</td>
<td>Deutsche Forschungsgemeinschaft</td>
</tr>
<tr>
<td>DF_H</td>
<td>Deposition fraction for humans (H = humans)</td>
</tr>
<tr>
<td>DF_T</td>
<td>Deposition fraction for animals (T = animals)</td>
</tr>
<tr>
<td>ECHA</td>
<td>European Chemicals Agency</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
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<tr>
<td>ELR</td>
<td>Elimination rate</td>
</tr>
<tr>
<td>ELRH</td>
<td>Elimination Rate for humans (H = humans)</td>
</tr>
<tr>
<td>ELRT</td>
<td>Elimination Rate for animals (T = animals)</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency (in the US)</td>
</tr>
<tr>
<td>GSD</td>
<td>Geometric standard deviation</td>
</tr>
<tr>
<td>HEC</td>
<td>Human Equivalent Concentration</td>
</tr>
<tr>
<td>HRTM</td>
<td>Human Respiratory Tract Model (developed by IRCP)</td>
</tr>
<tr>
<td>ICRP</td>
<td>International Commission on Radiological Protection</td>
</tr>
<tr>
<td>IIF</td>
<td>Interspecies interstitium factor</td>
</tr>
<tr>
<td>NEIR</td>
<td>interspecies normalisation and elimination rate ratio</td>
</tr>
<tr>
<td>NOAEC</td>
<td>No Observed Adverse Effect Concentration</td>
</tr>
<tr>
<td>LRT</td>
<td>Lower respiratory tract</td>
</tr>
<tr>
<td>MAK</td>
<td>Maximale Arbeitsplatzkonzentration (nonbinding OEL in Germany)</td>
</tr>
<tr>
<td>MMAD</td>
<td>Mass Median Aerodynamic Diameter</td>
</tr>
<tr>
<td>MoA</td>
<td>Mode of Action</td>
</tr>
<tr>
<td>MPPD</td>
<td>Multiple Pathway Deposition Model</td>
</tr>
<tr>
<td>MV</td>
<td>Breathing volume [m³/d]</td>
</tr>
<tr>
<td>NF</td>
<td>Normalisation factor</td>
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<tr>
<td>NFH</td>
<td>Normalisation factor for humans (H = humans)</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>---------</td>
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</tr>
<tr>
<td>NF&lt;sub&gt;T&lt;/sub&gt;</td>
<td>Normalisation factor for animals (T = animals)</td>
</tr>
<tr>
<td>NOAEC</td>
<td>No observed adverse effect concentration</td>
</tr>
<tr>
<td>NTP</td>
<td>National Toxicology Program</td>
</tr>
<tr>
<td>OECD</td>
<td>Organisation for Economic Co-operation and Development</td>
</tr>
<tr>
<td>OEHHA</td>
<td>Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency</td>
</tr>
<tr>
<td>OEL</td>
<td>Occupational exposure limits</td>
</tr>
<tr>
<td>PAR</td>
<td>Proximal alveolar region</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear neutrophils</td>
</tr>
<tr>
<td>PSLT</td>
<td>Poorly soluble and low toxicity (particle)</td>
</tr>
<tr>
<td>PSP</td>
<td>Poorly soluble particles</td>
</tr>
<tr>
<td>PU</td>
<td>Pulmonary region</td>
</tr>
<tr>
<td>TB</td>
<td>Tracheobronchial region</td>
</tr>
<tr>
<td>TCC</td>
<td>Total cell count</td>
</tr>
<tr>
<td>URT</td>
<td>Upper respiratory tract</td>
</tr>
<tr>
<td>VT</td>
<td>Tidal volume</td>
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1 Introduction

In the 1990ties, risk assessors developed a systematic procedure to derive a “human equivalent concentration” (HEC), starting from effect concentrations for the lower respiratory tract determined in rodent inhalation studies. For regulatory risk assessment on inhaled particles, HEC calculations have already been suggested for the general population in 1994 (US EPA, 1994). In 1999, based on deposition data of particles in experimental animals (e.g., Raabe et al., 1988) and humans (e.g., ICRP, 1994) and respective airway and airflow modelling (Yeh, 1980; Yeh and Schum, 1980), and starting from provisional versions (Anjilvel and Asgharian, 1995; Asgharian and Anjilvel, 1998) the US Chemical Institute of Toxicology (CIIT) in cooperation with the National Institute for Public Health and the Environment from the Netherlands (RIVM) developed a Multiple Pathway Deposition Model (MPPD) (RIVM, 1999). The HEC procedure included interspecies comparisons for a) deposition in the respiratory tract and b) retention, elimination, and clearance from the respiratory tract combined with several options for dose metrics (e.g. mass of particles or number of particles) and normalisation (e.g. to the lung surface area), all included in the MPPD software. However, it was also possible to limit the use of MPPD to deposition only (i.e. fraction of inhaled particles, which is deposited in a certain region of the respiratory tract) and supplement assumptions on elimination, dose metrics and normalisation from other sources as separate steps within the HEC calculation.

The HEC procedure including the MPPD deposition modelling has been used since 1999 mostly for specific areas of risk assessment of inhalation exposure to particles, with only few regulatory committees making use of this approach for standard setting: Systematic use of the HEC concept including MPPD dosimetry was established, e.g., by U.S. EPA for setting standards for the general population (US EPA, 2004) and as part of the derivation procedure for occupational exposure limits (OEL) in Germany (FoBiG, 2011). This German HEC approach has been presented in a guidance document on exposure risk relationship calculation for carcinogens in the lung (AGS, 2013), but is not limited to carcinogens. The HEC approach was, for example, used to derive an OEL for “poorly soluble, low toxicity” particles (PSLT) in Germany (Hartwig, 2012). However, this and subsequent applications in regulatory risk assessment induced some discussions on optimal parameter selection for MPPD modelling and on adequate procedures, e.g. for selecting dose metrics, normalisation and calculations of retained doses in the lung (e.g., Morfeld et al., 2015). Parts of the existing guidance on the German HEC procedure for workplaces (AGS, 2013) were found to be not sufficiently elaborated to guarantee unambiguous application. Moreover, a more recent version of MPPD (version 3.04) was released in 2016 and needs inclusion into an updated handling strategy. Progress in inhalation toxicology and new information on biokinetics of particles are to be considered.

This report provides study results, discussion, information, and example calculations to develop an updated guidance for HEC calculations for particles in the lower respiratory tract (LRT) for occupational exposure scenarios. Recent data will be reported and critically assessed. In most instances, no final conclusion on a generally agreeable default procedure will be possible within the framework of this report, as discussion on optimal use of the HEC approach and on its limitations is still ongoing;
respective arguments and data will be presented and suggestions for handling will be
provided, if regarded sufficiently qualified.
2 Definitions and Demarcation

2.1 Human Equivalent Concentration (HEC)

Within the context of this report the term “Human Equivalent Concentration (HEC)” will be used specifically to characterise the concentration of inhaled particles in the lower respiratory tract, where HEC is derived from rodent experimental data and biokinetic modelling according to the formula

\[ HEC = c_T \times \frac{AgV_T}{AgV_H} \times \frac{NF_H}{NF_T} \times \frac{ELR_H}{ELR_T} \times \frac{DF_T}{DF_H} \]

where

- \( T \) indicates animal data (“animal” in German language: “Tier”), \( H \) human data,
- \( c_T \) is the exposure concentration from the animal study, for which we want to know the human equivalent,
- \( AgV \) is the weighted breathing volume per day (German: “gewichtetes Atemvolumen”)
- \( NF \) is a normalising factor,
- \( ELR \) is the elimination rate, and
- \( DF \) is the deposition fraction.

This report will address all these ratios (\( AgV_T/AgV_H \) (Section 3), \( NF_H/NF_T \) (Section 5), \( ELR_H/ELR_T \) (Section 6), and \( DF_T/DF_H \) (Section 4)) separately in order to discuss procedures to quantify each of them (see Sections 3 to 6, for details). Finally, the aggregate procedure and the results for the ratio HEC/c_T will be discussed in more detail in Section 7.

Below, we will only provide a standard quantification procedure for HEC and for the single terms and ratios within this calculation: this will be called “default HEC calculation”. We will therefore define, e.g., particles properties and exposure conditions, for which default HEC can be calculated. For example, in this report a default HEC calculation is limited to a certain range of micro-sized particles only (Section 2.2). Therefore, if a HEC is to be quantified for nanoparticles, this may also be possible, but this is not regarded a default HEC calculation. Non-standard (non-default) interspecies extrapolation of human equivalent concentrations is not discussed in this report.

The procedure to calculate HEC in the way as described by the formula given above, we will call the “4 ratios approach” (product of 1. \( AgV_T / AgV_H \), 2. \( NF_H / NF_T \), 3. \( ELR_H/ELR_T \), 4. \( DF_T/ DF_H \)), in order to discriminate it from the “aggregate 3 ratios approach”, which is alternatively suggested in Section 7.4 for HEC-calculations.
2.2 Particle properties

The HEC default procedure as discussed in this report is limited to particles sizes with a mass median aerodynamic diameter (MMAD) or agglomeration diameter for nanoparticles of 0.5 - 2 µm (for justification see Section 4.8). The more general HEC procedure is linked to the respirable particle fraction and covers a broader range. The most recent MPPD software (version 3.04) provides means to calculate HEC within a particle size range from 0.01 µm to 10 µm, i.e. a considerably larger range than is covered by the default procedure. However, consequences for interspecies calculations for particle sizes beyond the mentioned smaller applicability range have to be discussed case-by-case and are not covered below. Specifically, for nanoparticle-specific transport and deposition, MPPD provides a separate adapted model for particles with a size of less than 0.1 µm, which is not addressed in this report. Particles are assumed to be of spherical shape. Fibres are not covered. For particles or agglomerates with irregular shape, MPPD provides an “equivalent diameter model” and for fibres with an aspect ratio\(^1\) larger than 3, different dosimetry assumptions are provided, but not addressed below.

The HEC default procedure is not limited to specified widths of the particle size distributions (defined by the standard deviation of MMAD), which means that it is not restricted to monodisperse or polydisperse particle distributions. However, no systematic testing of the uncertainties from wide distributions has been performed so far. It is assumed that studies with standard deviations of > 1.3 are not adequate for default HEC calculations, if the corresponding MMAD is close to the upper or lower applicability range (i.e. close to 0.5 µm or 2 µm).

Also, the HEC default procedure is not limited to a specific bioaccessibility\(^2\) of particles in the respiratory tract. The procedure thus covers poorly and highly soluble compounds. However, specific uncertainties have to be addressed for highly water-soluble particles (see Sections 4.6, 5.5, 6.6, and 7.4). The HEC procedure, as discussed below, is linked to solid dry particles and does not address liquid aerosol exposure. The assessor should be aware of potentially differing solubility of particles in physiological lung fluids (i.e. epithelial lining fluid, interstitial fluid, lysosomal fluid), as those may influence mode of action, elimination and corresponding adequate normalisation (Sections 5.5, 6.6 and 7.4).

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1. aspect ratio of a geometric shape is the ratio of its sizes in different dimensions
2. For the purpose of this discussion of respiratory effects, the term „bioaccessibility“ is preferred to „bioavailability“, as bioavailability usually refers to systemic (not to local) biological availability. However, some authors cited below use the term of bioavailability also for solubility in local respiratory fluids.
2.3 Effects in the upper respiratory tract

MPPD deposition calculations also report deposition in the upper respiratory tract (URT). Respective calculations point to significant species differences and should possibly be considered, if the critical respiratory effect of a particle is in the URT (e.g. Shang et al. (2015)). Apart from MPPD also other deposition models directly address the URT (e.g. Morris et al., 2010; Moss, 2010). However, modelling of particle deposition in the URT is not further discussed below.

For gases and vapours with respiratory effects the URT often is the critical target. Asgharian et al. (2012) developed corresponding models, e.g. for formaldehyde, acrolein and acetaldehyde. These models also include dosimetry for the URT and the LRT region. However, this report is limited to particles. It is regarded worthwhile and relevant, to provide a concept for HEC calculations in the URT in future.

2.4 Nanoparticles

Specific conditions of nanoparticle HEC calculations are not addressed in this report. One reason for this is the applicability domain (particle size range) for this default approach (see Section 2.2). However, HEC calculations for agglomerates of nanoparticles with sizes above 0.5 µm are covered below.

It should be noted that workplace exposure usually includes only small fractions of single nanoparticles or agglomerates < 0.1 µm. There may be exemptions like welding fumes (Stebounova et al., 2018), for which a separate discussion is necessary (not addressed in this report). Numerous studies were performed with nanoparticles or agglomerates with smaller sizes than 0.1 µm; a significant part of those were studied under in vitro conditions. It is currently not suggested that such nanoparticles are to be handled as separate entity with significantly different properties from larger bulk particles (Gebel et al., 2014).
3 Weighted Breathing Volume: $\text{AgV}_T/\text{AgV}_H$

3.1 Breathing volume comparisons

Averaged weighted breathing volumes ($\text{AgV}$) (with the unit: air volume in m$^3$ per day) have to be multiplied with workplace particle air concentrations in order to determine the absolute amount of particles inhaled per day. The ratio $\text{AgV}_T/\text{AgV}_H$ provides interspecies differences with respect to breathing volumes.

It should be noted that breathing volume also influences the deposition fraction in subsequent calculations (Section 4). Therefore, it is suggested that identical data and quantification procedures are applied a) to calculate $\text{AgV}_T/\text{AgV}_H$ and b) as input to MPPD deposition modelling.

3.2 Current quantitative approach in Germany

In Germany, the HEC approach is currently used for interspecies extrapolation within the framework of deriving OELs for particulate substances (AGS, 2013). Under chronic exposure conditions in a rat study with exposure for 6h/d the breathing volume is calculated as follows (AGS, 2013):

$$\text{AgV}_T = \text{tidal volume} \ [\text{mL/breath}] \times \text{breathing frequency} \ [\text{breaths/min}] \times 60 \text{ min/h} \times 6\text{h/day}$$

$$= 2.1 \text{ mL} \times 102 \text{ 1/min} \times 60 \times 6\text{h/d} = 77 \text{ L/d} = 0.077 \text{ m}^3/\text{d}$$

with a default tidal volume of 2.1 mL/breath and a breathing frequency of 102 breaths/min. (0.214 L/min/rat). These values are from Long-Evans rats (Mauderly et al., 1979), but have been applied for any rat strain.

If exposure in the chronic rat study was at 5 days per week only, the average long-term $\text{AgV}_T$ is calculated as follows (FoBiG, 2011; Hartwig, 2012):

$$\text{AgV}_T = 0.077 \text{ m}^3/\text{d} \times 5/7 = 0.055 \text{ m}^3/\text{d}.$$

For chronic human exposure an average human breathing volume of 10 m$^3$/d is assumed, meant to stand for the breathing volume under light physical activity. This value is averaged over longer periods to calculate a yearly average $\text{AgV}_H$: (assuming exposure at 240 days per year):

$$\text{AgV}_H = 10 \text{ m}^3/\text{d} \times 240 \text{ d/365 d} = 6.57 \text{ m}^3/\text{d} \ (\text{FoBiG}, \ 2011; \ \text{Hartwig}, \ 2012).$$

Therefore, in the current HEC default approach in Germany the ratio is set as follows:

$$\text{AgV}_T/\text{AgV}_H = (0.055 \text{ m}^3/\text{d})/(6.57 \text{ m}^3/\text{d}) = 0.008.$$
The weekly exposure of the experimental animals may be 5 days per week, or, sometimes, 7 days per week. According to OECD 413 (90-day inhalation toxicity study) animals are typically exposed at 5 days per week. However, exposure at 7 days per week is also possible.\(^3\) In contrast, OECD 452 (chronic toxicity testing) assumes exposure at 7 days per week, but would accept exposure at 5 days per week, if justification is provided.\(^4\) If exposure was at 7 days per week the calculation of the AgVT/AgVH - ratio should be modified accordingly, i.e. 0.077 m\(^3\)/d should be used.

Identical tidal volumes and breathing frequencies are used in MPPD, version 2.11: for rats, the default tidal volume is set to 2.1 mL and the breathing frequency to 102 breaths/minute. For 8 hours human exposure during workdays a breathing frequency of 20 breaths/minute and a tidal volume of 1040 mL is used as current default, resulting in a daily breathing volume of 20 x 60 x 8 x 1040 = 9,984,000 mL ≈10 m\(^3\)/day. For further discussion see Section 4.

There is a limitation of the current approach in Germany: Default breathing volume values are derived from only one rat strain (Long-Evans rats). Therefore, refinement is needed to cover breathing volumes for other rat strains (Section 3.3).

### 3.3 New data

No new relevant data on human breathing volumes (AgVH) have been found in recent literature.

MPPD 3.04 permits the use of “Long-Evans rat” data for breathing frequency and tidal volume in experimental animals (rat). The data from Long-Evans rats discussed above (breathing frequency: 102/minute; tidal volume: 2.1 mL) are maintained and can also be used to calculate AgVT. However, if specific body weights are provided and/or if other rat strains were used, MPPD 3.04 applies an allometric formula by Miller et al. (2014; 2013) to calculate breathing frequency and tidal volume for a given body weight. For example, for Sprague-Dawley rats (nose- or head-only-exposure) the following allometric formula is used in MPPD 3.04:

\[
\text{Tidal Volume (VT) [mL]} = 1000 \ast (-0.060911+0.0013795\ast BW)/166 \text{ (Miller et al., 2014),}
\]

where BW is “body weight” in grams and 166 is a default value for breathing frequency of Sprague-Dawley rats, irrespective of body weight.

There are no clear rules provided in MPPD 3.04 how to calculate tidal volumes for other strains of rats but Sprague-Dawley or Long-Evans. Therefore, the Sprague-Dawley formula in combination with the specific body weight will be applied for any tested rat strain, if one uses the default automatic procedure of MPPD 3.04. See Section 4.3.2 for further discussion of the MPPD 3.04 calculation procedure and template. However, another allometric formula has recently been published by the

\(^3\) https://www.oecd-ilibrary.org/environment/test-no-413-subchronic-inhalation-toxicity-90-day-study_9789264070806-en

\(^4\) https://www.oecd-ilibrary.org/environment/test-no-452-chronic-toxicity-studies_9789264071209-en
Californian Office of Environmental Health Hazard Assessment (OEHHA, 2018). The OEHHA regression formula has been derived from a large set of data from different rat strains including but not limited to male and female F344-rats, Wistar rats, Long-Evans rats and Sprague-Dawley rats. This regression results in an **Inhalation Rate (I)**:

\[ I = 0.702 \times BW^{2/3} \text{ (unit: m}^3\text{/day)} ,\]

which could directly be used for AgV\(_T\)-calculation. This formula is linked to environmental exposure (24 h/d); for occupational exposure the value needs to be divided by 4 (if the study was performed with 6 hrs/day, which is the typical experimental design).

If the regression formula by OEHHA is used with a body weight of 250 grams a daily breathing volume of **0.28 m}^3\text{/day}** is provided (blue bold round mark, Figure 3-1). If the regression formula by Miller et al. is used (MPPD 3.04, Sprague Dawley Rat, 250 grams, asymmetric), those defaults lead to a daily breathing volume of **0.41 m}^3\text{/day}** (green bold round mark, Figure 3-1; \( VT = 1.71 \text{ mL; breathing frequency} \ 166 \times 60 \times 6 = 59760 \text{ breaths/ 6 hrs; } 1.71 \times 59760/1000000 = 0.102 \text{ m}^3/6 \text{ hrs; breathing volume/24h} = 0.102 \times 4 = 0.41 \text{ m}^3/\text{d} )). The original calculation in the German procedure and the default procedure in MPPD 2.11 (see Section 3.2) results in **0.31 m}^3\text{/day}** (red bold round mark, Figure 3-1; for 24 hrs. exposure, \( 0.214 \times 60 \times 6 \text{ h/d} = 77 \text{ Liters/ d} = 0.077 \text{ m}^3/6 \text{ h} \times 4 = 0.31 \text{ m}^3/\text{d}) . This does not mean that either the breathing volume of 0.31 m}^3\text{/day} (current default) or the 0.41 m}^3\text{/day} (MPPD 3.04 for Long-Evans using the Miller formula) were incorrect, but it shows that the Miller et al. regression and the OEHHA-regression differ considerably. From this presentation it is also obvious that breathing volume calculations include high variability and high uncertainties. Similarly, MPPD 3.04 (ARA, 2018, online - help-handbook) confirms that there is “considerable variability in published measurements of breathing frequency and tidal volumes”.


Figure 3-1: Inhalation rate per day (24h) as derived by OEHHA (2018) (green line); for interpretation of bold round marks (red, green, blue) (inserted roughly from graphical scale) see text, above. [note that MV (m³/day) is described as breathing volume per day by the authors, not as breathing minute volume]; figure adopted with permission from OEHHA (2020; personal communication; April 8th, 2020; modified by inserted coloured bold round marks)

3.4 \( \text{AgV}_T / \text{AgV}_H \) from mice data

ECHA (2018) guidance provides default values for “inhalation volume/ hour” for (male) mice with 2.5 liters (default body weight: 30 grams). This corresponds to a breathing minute volume of 41.66 mL/min\(^5\). If breathing parameters are taken from MPPD 3.04 for a 30g mouse (BALB/c or B6C3F1), a default of 296.4 breaths per minute and a tidal volume of 1.799 mL are presented as default in the respective template for exposure. This tidal volume is regarded as incorrect.

The MPPD-manual for version 3.04 provides allometric formulae for breathing frequency and tidal volume:

\(^5\) Table R.8-17;ECHA R.8 (2018)
Breathing frequency (BF) [breaths/min] = 65.58 x BW \(^{-0.4275}\) (BW in kg) and
Tidal volume (VT) [mL/breath] = 0.64175 x BW\(^{0.29398}\) (BW in kg).

If BW = 0.03 kg are used for calculation, this results in a breathing frequency (BF) of 296 breaths per minute (confirming the default from MPPD template), but VT calculation results in a tidal volume of 0.229 mL, which is different from 1.799 mL (as documented in the MPPD 3.04 template). The product of BF x VT = 67 mL/min (breathing minute volume), which is only moderately different to the default from ECHA (2018), i.e., 41.66 mL/min (ECHA) vs. 67 mL/min (via allometric formula, as documented in MPPD 3.04-manual) (ARA, 2018). Snipes (1989) provides a slightly lower breathing minute volume of 40 mL/min for mice.

The values in the MPPD 3.04 manual are also supported by Miller et al. (2016), who report 144-388 breaths per minute for mice (296 breaths are within this range) and a tidal volume of 0.218 mL for a 20 gram mouse (which is close to 0.229 mL from allometric calculation for the 30g mouse).

Hsieh et al. (1999) provides a different calculation formula to calculate a breathing volume (MV):

$$MV = 0.37 \text{ BW}^{1.36} [\text{m}^3/\text{d}]$$ and Tidal volume = 0.0023 BW\(^{1.36}\) (BW = body weight in grams).

For a 30g mouse, this results in a tidal volume of 0.235 mL (which, again, is very similar to the allometric calculation provided in the MPPD 3.04 manual). However, the breathing minute volume of 37.76 mL/min is quite low due to a low breathing frequency of 161 breaths per minute assumed in this calculation.

Kolanjiyil et al. (2019) report some data on breathing patterns for mice “at rest” or with “light exertion”. Mass flow rate is noted to be 66-125 mL/min, tidal volume between 0.2 and 0.22 mL and breathing frequency between 332 and 572 breaths per minute. As experimental animals are usually exposed at resting exposure conditions, the lower end of this range (i.e., 332 breaths per minute) can be used for further calculations. This is close to the default in MPPD 3.04.

An overview of the breathing volumes derived from above sources is provided in Table 3-1.

Note, that the breathing volume per day assumes 6 hours daily exposure, which is in agreement to the standard exposure duration in experimental animal studies.
Table 3-1: Daily breathing volume [m³/6h] for mice, different sources

<table>
<thead>
<tr>
<th>Source</th>
<th>BW&lt;sub&gt;mouse&lt;/sub&gt; [g]</th>
<th>Breathing frequency per minute [bpm]</th>
<th>Tidal volume (VT) [mL]</th>
<th>Breathing vol. (m³/6h)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECHA (2018)</td>
<td>30</td>
<td></td>
<td></td>
<td>0.015</td>
<td>VT and bpm not reported</td>
</tr>
<tr>
<td>MPPD 3.04 calculation sheet</td>
<td>30</td>
<td>296.4</td>
<td>1.799</td>
<td>0.192</td>
<td>Values suggested for default. Calculated VT is questioned in this analysis. Different values can be entered in calculation sheet</td>
</tr>
<tr>
<td>MPPD 3.04 online manual</td>
<td>30</td>
<td>296</td>
<td>0.229</td>
<td>0.024</td>
<td>Different BW can be calculated (allometric formula)</td>
</tr>
<tr>
<td>Snipes (1989)</td>
<td>30</td>
<td></td>
<td></td>
<td>0.014</td>
<td>Calculated from minute volume of 0.04 L/min.</td>
</tr>
<tr>
<td>Miller et al. (2016)</td>
<td>20</td>
<td>144-388</td>
<td>0.218</td>
<td>0.011-0.03</td>
<td>Only range provided; note the BW of 20 grams</td>
</tr>
<tr>
<td>Hsieh et al. (1999)</td>
<td>30</td>
<td>161</td>
<td>0.235</td>
<td>0.0136</td>
<td>Different BW can be calculated (allometric formula)</td>
</tr>
<tr>
<td>Kolanjiyil et al. (2019)</td>
<td>?</td>
<td>332 (rest)-572 (light exertion)</td>
<td>0.2-0.22</td>
<td>0.024-0.045</td>
<td></td>
</tr>
</tbody>
</table>

We suggest to use the MPPD 3.04 manual allometric formulae to calculate the daily breathing volume for mice. When the deposition factor is derived by applying MPPD 3.04 (Section 4.11), the default value in the exposure template needs to be corrected to the value as derived by the allometric equation, because the template default value is apparently too high. Defaults need to be agreed by further discussions and considerable variability in breathing rates and subsequent breathing volumes should be noted. If, for example, a breathing volume of 0.024 m³/day in the mouse is taken from Table 3-1, this should be transformed by a factor of 5/7 (average chronic daily exposure) to get $\text{AgVT}$ for mice: $0.024 \text{ m}^3/\text{day} \times 5/7 = 0.017 \text{ m}^3/\text{day}$ (if the experimental chronic exposure is five days per week).

In Section 3.2, we derived the default breathing volume for human workplace exposure ($6.57 \text{ m}^3/\text{d}$; weighted daily exposure).
With a weighted breathing volume of 0.01714 m³/day in the mouse study, a weighted breathing volume interspecies ratio (AgVT / AgVH) for mice is calculated

$$\frac{AgVT}{AgVH} = \frac{0.017 \text{ [m}^3\text{/day]} }{6.57 \text{ [m}^3\text{/day]}} = 0.0026$$

This default factor needs to be adapted to the specific body weight of the mice in the respective experimental study. This value is about threefold lower than the respective default value for rats.

### 3.5 Conclusions

From analysing updated breathing volume data in animals and, to a limited extent, in humans, we conclude:

- Human default assumptions on AgVH have been maintained as in former assessments with a breathing frequency of 20/min and a tidal volume of 1040 mL, resulting in an inhalation breathing volume of 10 m³/d, which represents light physical activity. When averaged over chronic exposure periods (240 days per year) this results in a value of 6.57 m³/d. However, maintaining the well-established default of 10 m³/d, means relevant simplification. In reality, different breathing patterns (e.g., mouth vs. nose breathing) and individual differences in exercise and physiognomic parameters lead to a relevant range of AgVH-values and, therefore, increase AgVT/AgVH-variability (not further assessed in this report).

- For experimental data on breathing volumes of rats, a high variability is documented; existing allometric regression formula result in different breathing volumes at identical body weights. The range of the ratio AgVT/AgVH is 0.0076-0.024. Therefore, the current default value of 0.008 for AgVT/AgVH apparently is a conservative approach.

- However, we propose to substitute the fixed value of 0.008 by a flexible value according to an allometric calculation.

- We suggest, not to switch to the OEHHA allometric breathing volume calculation because:
  - The OEHHA-formula is less conservative for large body weights than the MPPD 3.04 formula
  - It is more complicated to use those OEHHA-derived values in combination with MPPD 3.04 software. The use of different breathing volume rates in MPPD 3.04 and for the standardized breathing value factor should be avoided.

- Therefore, the use of the flexible MPPD 3.04 generated values are proposed for AgVT calculation, which can also be calculated manually by
  - Breathing rate (166 breaths per minute) x 60 Minutes x 6 hours = 59670 breaths per working day
  - Tidal Volume [m³] = (-0.060911+0.0013795*BW)/166000 (Miller et al., 2016),

- Deviations from this default calculation procedure should be considered,
If explicit data are available for breathing frequency and/or tidal volume and/or breathing volumes are directly available from the experimental study,

- if exposure of experimental animals were not “nose- or head-only”, but “whole body”,
- if the human exposure scenario deviates from the default (i.e. 10 m³/d breathing volume and exposure for 240/365d/year)

For AgVT, in either of those non-default cases, the Help-Handbook-Online MPPD 3.04 provides supplemental allometric calculation formulae (note that those are linked to 24hrs exposure).

- If interspecies AgVT/AgVH is to be calculated from mice data, allometric formulae from MPPD 3.04 should be used for calculation of the animal breathing volume. The resulting interspecies factor for AgVT/AgVH is 0.0026 for a 30 gram - mouse to the human occupational scenario.

Table 3-2: Example calculations of weighted breathing volumes and AgVT/AgVH ratios for rat/human interspecies comparisons

<table>
<thead>
<tr>
<th>Body weight (rat)</th>
<th>AgVT</th>
<th>AgVH</th>
<th>AgVT/AgVH</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not assigned</td>
<td>0.055</td>
<td>6.57</td>
<td>0.008</td>
<td>Current default in Germany (AGS, 2013; FoBiG, 2011)</td>
</tr>
<tr>
<td>250 g</td>
<td>0.07</td>
<td>6.57</td>
<td>0.01</td>
<td>MPPD 3.04 (allometric formula by Miller et al. (2014))</td>
</tr>
<tr>
<td>250 g</td>
<td>0.05</td>
<td>6.57</td>
<td>0.0076</td>
<td>OEHHA (2018) (allometric formula by OEHHA)</td>
</tr>
<tr>
<td>500 g</td>
<td>0.08</td>
<td>6.57</td>
<td>0.012</td>
<td>500g (example default body weight for male rats, documented in ECHA (2018; Table R.8-17) MPPD 3.04 (allometric formula by Miller et al. (2014))</td>
</tr>
<tr>
<td>500 g</td>
<td>0.16</td>
<td>6.57</td>
<td>0.024</td>
<td>500g (example default body weight for male rats, documented in ECHA (2018; Table R.8-17) (allometric formula by OEHHA, 2018)</td>
</tr>
</tbody>
</table>
4 Deposition fraction (DF_T/ DF_H)

4.1 Deposition fraction – overview

Due to the specific anatomy and due to differences in air flow in the respective species, significant differences in deposited doses in the lower respiratory tract (LRT) exist between rodents and humans. Therefore, external exposure (corresponding to ambient air concentration) is regarded as a poor starting point for interspecies comparisons; the deposited dose in the pulmonary or total lower respiratory tract region may be more adequate. Therefore, the ratio of the deposition fractions is included in the HEC calculation procedure.

The term *fraction* within “deposition fraction” relates to the external (ambient air) exposure concentration (percent/100). However, in MPPD calculations the fraction can also be related to the “inhalable” particle concentration. This correction with respect to inhalability in calculations (“inhalability adjustment”) is discussed, when the influence of particle size is presented in a broader context (Section 4.9).

Currently, modelling of the deposited dose is an integrated element of MPPD. Some other modelling approaches are briefly mentioned in Section 4.2. For the German workplace HEC calculation procedure MPPD is applied and the calculation of deposition with this software is presented in more detail below (Section 4.3).

In guidances for interspecies comparisons with respect to particle effects in the LRT it is not precisely defined, whether deposition should be averaged for the total LRT or whether reference to local or regional area deposition within the LRT would be more adequate. This discussion is subdivided in 2 parts: discriminating the pulmonary region from the tracheobronchial region in Section 4.4, and discriminating average regional deposition from local “hot spots”, which leads to high inhomogeneity (Section 4.5).

Usually, solubility of particles is only discussed in the context of retention (because soluble particles are usually eliminated much faster from the lung). However, solubility of particles may also influence deposition patterns, as is documented in Section 4.6. Deposition in the lung is influenced not only by particle size but also by particle density. In interspecies comparisons, it is relevant to know whether the relative fractional deposition ratio (rodents/humans) changes depending on size (Section 4.8) and/or density (Section 4.7) within the applicability range for default calculations.

DF_T/ DF_H output from MPPD is a single ratio. However, in reality for both species (rodents and humans) there may be significant variability in the respective deposition fraction. This aspect is further discussed in Section 4.10.

Most interspecies extrapolations (and therefore HEC-calculations) are based on rat studies. However, MPPD also permits to calculate the deposition for mice (Section 4.11). Finally, conclusion from the discussed dimensions of deposition and the fractional deposition in rodents vs. men (DF_T/ DF_H) are presented in Section 4.12.
4.2 Alternative models for deposition modelling

Dosimetry modelling for the respiratory tract has been developed in the 1990th years, e.g. by the U.S. EPA, using the term “regional deposited dose ratio” (RDDR) with specific approaches for the upper and the lower respiratory tract (US EPA, 1994). Within interspecies extrapolation, RDDR is used to adjust the animal deposited dose to a human-equivalent concentration (HEC). The RDDR software does not provide estimation of particle clearance or retention and the use of this approach has decreased over time (Kuempel et al., 2015).

Also, in the 1990th, the MPPD model has been developed (Anjilvel and Asgharian, 1995; Asgharian and Anjilvel, 1998; Asgharian et al., 2001; Price et al., 2002), which is discussed in more detail in Section 4.3. Overviews on similar modelling approaches including and in addition to MPPD are provided, e.g. by Isaacs et al. (2005), Kuempel et al. (2015), Fröhlich et al. (2016), and Lejon (2019).

For human exposures, the International Commission on Radiological Protection (ICRP) and the National Council on Radiation Protection and Measurements (NCRP) have, independently from each other, developed respiratory tract models for the use in radiation protection. However, these deposition data can also be applied for non-irradiant particles. Those models differ from the modelling within MPPD with respect to the mathematical model (NCRP/ICRP: semi-empirical; MPPD: deterministic) and lung geometry (NCRP/ICRP: symmetric lung geometry; MPPD: 5-lobe symmetric for default) (Asgharian, 2018). The NCRP model (1997) gives rather similar results as the ICRP model (1994), but significant differences were found for nano-sized particles, where ICRP does not account for enhanced diffusional deposition (Yeh et al., 1996).

The ICRP-model is also described as Human Respiratory Tract Model (HRTM) and has been modified over the years (Gregoratto et al., 2010; Kuempel et al., 2001). Even though those models for human exposure are rather similar with respect to deposition, observations from workers still demonstrate deficits with respect to clearance (Kuempel et al., 2015) and therefore are currently updated (Bailey et al., 2007)\(^6\). These deficits are further discussed in Section 6.4 (retention and clearance).

All the models discussed above are validated mostly with poorly soluble particles. However, due to hygroscopic properties, deposition patterns may differ significantly for water-soluble particles. Winkler-Heil (2014) proposed deposition modelling specifically for hygroscopic particles, but, up to now, there is no user-friendly software available for routine application. The principles of a deposition model for hygroscopic particles have been described by Ferron et al. (2013)\(^7\). More details on the influence of solubility on deposition is provided in Section 4.6.

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\(^6\) No information on a realisation of this intended update is available
\(^7\) Update information and a preliminary calculation sheet are available from [https://www.helmholtz-muenchen.de/cma/forschung/topic-iii-aerosol-physik/projekte/index.html](https://www.helmholtz-muenchen.de/cma/forschung/topic-iii-aerosol-physik/projekte/index.html) -> check for term: „Lung Deposition Model“ and [https://www1.helmholtz-muenchen.de/ioec/lung-deposition/hpldb06_i/index3i.php](https://www1.helmholtz-muenchen.de/ioec/lung-deposition/hpldb06_i/index3i.php)
4.3 MPPD deposition modelling

4.3.1 MPPD Version 2.11 vs. Version 3.04

Applied Research Associates (ARA) issued an updated version 3.04 of the Multiple Path Particle Dosimetry Model in 2016, which is more closely described by Miller et al. (2016), Asgharian et al. (2014) and the online MPPD-Help-Handbook (ARA, 2018). A number of changes and improvements compared to the former version 2.11 are included:

- Deposition modelling is now provided not only for the rat, but additionally also for B6C3F1 and Balb/c mice, male rhesus monkeys, sheep, and pigs.
- For the rat, deposition modelling is extended from Long-Evans rats only to Sprague-Dawley rats, where optional adjustments to different body weights can be considered and different modelling approaches (symmetric or asymmetric airway modelling) can be selected.
- Further differentiated assessments for specific lung areas are possible, as more data on local alveolar surfaces are integrated.
- Further, allometric calculation procedures are provided for, e.g., functional residual capacity (FRC) and upper respiratory tract deposition.
- Modelling of deposition for toxic substances adhered to other particles is made possible (e.g. for environmental cadmium exposure associated with particulate matter).
- Deposition of particles with multimodal size distributions can be calculated.
- Lymph node clearance is integrated as an elimination route.
- The applicability domain of the model is increased to a particle size range of 0.001 µm to 100 µm.
- Additional specific optional human exposure scenarios (like children’s exposure profiles) can be modelled.

As MPPD is used for various scenarios, some of the recent changes may be very helpful, e.g., for site specific environmental risk assessments, but are most likely less important for interspecies extrapolation for regulatory standard setting. Note that MPPD is not always used for all steps of the HEC calculation. For example, in the German procedure, it was decided to assess interspecies differences in clearance not with MPPD (see Section 6.1).

4.3.2 MPPD (Version 3.04) application

No specific guidance on how to use MPPD (version 3.04) is included in this report. However, a few remarks on application of this software are useful to ensure identical results. In most cases, required input is identical to the earlier version (MPPD version 2.11). However, some modifications are summarized below:

- For step: “Input data, airway morphology, rat” a specific choice is added in the field: “Model”, where “Asymmetric (lung model) for Sprague Dawley (rat)” can be selected. If agreed, the user is asked to provide a body weight in grams.

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(output is erroneously given in kilograms). This will automatically change figures for FRC and URT due to allometric scaling (Please change number presentation from "decimal comma" to "decimal point", as the program will turn to default, if you miss this correction).

- For step “Input data, inhalant properties, aerosol”, select “Inhalability adjustment” (yes), for either species (e.g. rat, humans) (for discussion see Section 4.9). Be sure to enter specific values for density and diameter (mostly given by MMAD). For closer discussion on the influence of density and particle size on deposition see Sections 4.7-4.8. For standard setting in default procedures do not change “aspect ratio”, single vs. multiple or multimodal, and do not mark for “equivalent diameter model”.

- For step “Input data, exposure scenario, constant exposure”, note that, for experimental animals, breathing frequency and tidal volume will be automatically adjusted to the body weight of the animal you have entered above (“Input data, airway morphology, rat”). But manual changes are permitted in non-default assessments. For humans, enter identical changes as requested in version 2.11 (e.g. for workplace exposure scenario: breathing frequency 20/minute and tidal volume 1040 mL, oronasal-normal-augmenter). Do not modify the standard inserted values for “acceleration of gravity”, “upright” body orientation, “inspiratory fraction” and “pause fraction”. For rodent input data, switch to “nose only exposure”, if applicable.

### 4.3.3 Quantitative changes (MPPD 3.04 vs. MPPD 2.11)

A quantitative comparison of the results between MPPD 2.11 and MPPD 3.04 has been performed. We analysed the influence of body weight and corresponding breathing volume/d on deposition fractions and on the ratio of the deposition fractions (DF_T/DF_H ratio) of the two versions. We compared results, when a) the allometric formula from either OEHHA (2018) for breathing volume was used or when b) the allometric formula from Miller et al. (2014) was applied. The latter approach is the default approach suggested in MPPD 3.04 (figures for tidal volume and breathing frequency automatically generated for a given body weight of rats). The former approach needs manual input of the tidal volume and the breathing frequency corresponding to the OEHHA calculation of the breathing volume/day (Section 3.3).

- Table 4-1 shows the output of MPPD 3.04 vs. MPPD 2.11 without consideration of the specific rat body weight.
- Table 4-2 shows the output of MPPD 3.04 vs. MPPD 2.11, where the specific rat body weight is considered (only possible in version MPPD 3.04) and the OEHHA calculation is used for the breathing volume.
- Table 4-3, finally, shows the output of MPPD 3.04 vs. MPPD 2.11, where the specific rat body weight is considered (only possible in version MPPD 3.04) and the standard calculation suggested by MPPD is used for the breathing volume.

For all calculations deposition fractions in the pulmonary region (Alv) and/or in the tracheobronchial region (TB) are shown.
Without changes for body weight, for this example the results of the two versions were close to identical (Table 4-1).

However, if the specific body weight of F344 rats is taken into account (only applicable in MPPD 3.04, not in MPPD 2.11) and if breathing volume is calculated by OEHHA allometric regression, this results in relevant changes, in case only the pulmonary deposition or deposition in the TB region is assessed (Table 4-2). There is no relevant change, if deposition in the total LRT (TB+Alv) is considered (DFT/DFH: 0.45 in MPPD 3.04; 0.49 in MPPD 2.11). However, if only the pulmonary region is addressed, the difference increases (DFT/DFH: 0.32 in MPPD 3.04; 0.49 in MPPD 2.11). Note that even though the absolute deposition fraction is low in the TB area (1.66 or 2.82 percent, respectively), high DFT/DFH ratios can result if only the TB region is considered (0.47 or 0.8, respectively; Table 4-2). Similar ratios are observed, when the MPPD default breathing volume calculation is used instead of the OEHHA formula (Table 4-3).

Further, similar calculations indicate that the ratio DF\textsubscript{T}/DF\textsubscript{H} may be different up to about a factor of 2 (also for the pulmonary region only), depending on the input data, body weight and derived breathing volume and the specific region of the LRT addressed.

Table 4-1: MPPD comparative calculations (version 3.04 vs. 2.11); input data: MMAD: 1.4 µm; GSD: 2.1; Density: 2.0 g/cm³; concentration: 0.067 mg/m³, default body weight assumptions accepted, parameters entered as requested in ERR-guidance (AGS, 2013)

<table>
<thead>
<tr>
<th>Depos. fraction</th>
<th>MPPD 3.04</th>
<th>MPPD 2.11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TB+Alv</td>
<td>TB</td>
</tr>
<tr>
<td>Human</td>
<td>0.1298</td>
<td>0.0354</td>
</tr>
<tr>
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<td>0.0162</td>
</tr>
<tr>
<td>DF\textsubscript{T}/DF\textsubscript{H}</td>
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<td>0.46</td>
</tr>
</tbody>
</table>
Table 4-2: MPPD comparative calculations (version 3.04 vs. 2.11); input data: MMAD: 1.4 µm; GSD: 2.1; Density: 2.0 g/cm³; concentration: 0.067 mg/m³, experimental body weight 434 grams F344-rat (NTP-study data; MPPD 3.04 only), breathing frequency: 140 (Mauderly et al. (1979) for F344-rats); Tidal volume as from OEHHA (2018) allometric formula associated with breathing volume at a given breathing frequency (see Section 3.3)

<table>
<thead>
<tr>
<th>Depos. fraction</th>
<th>MPPD 3.04</th>
<th>MPPD 2.11</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>DFₜ/DFₜ</td>
<td>0.45</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Table 4-3: MPPD comparative calculations (version 3.04 vs. 2.11); input data: MMAD: 1.4 µm; GSD: 2.1; Density: 2.0 g/cm³; concentration: 0.067 mg/m³, experimental body weight 434 grams F344-rat (NTP-study data; MPPD 3.04 only), breathing frequency: 166 (default SD-rats; MPPD 3.04); Tidal volume, default for body weight in MPPD 3.04 (see Section 3.3)

<table>
<thead>
<tr>
<th>Depos. fraction</th>
<th>MPPD 3.04</th>
<th>MPPD 2.11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TB+Alv</td>
<td>TB</td>
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<tr>
<td>Human</td>
<td>0.1298</td>
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<tr>
<td>Rat</td>
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<td>0.0315</td>
</tr>
<tr>
<td>DFₜ/DFₜ</td>
<td>0.48</td>
<td>0.89</td>
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</tbody>
</table>

An additional difference between MPPD versions was observed, when we analysed DFₜ or DFₜ values in relation to particle sizes (Section 4.8). Earlier versions of MPPD (i.e. versions 2.01 or 2.11) have shown minimum deposition at about 0.5 µm of diameter, with some increase in deposition with larger particle sizes and a local maximum at ≈ 2 µm (MPPD 2.11) or ≈ 3 µm (MPPD 2.01) (Figure 4-4). This local maximum was not observed with MPPD version 3.04, instead a monotonic decline of DFₜ with increasing particle sizes occurs (Figure 4-1 and Figure 4-3). For human data, MPPD 3.04 still demonstrates such a local maximum for low density particles (Figure 4-3), but not for high density particles (Figure 4-1). Consequences of these differences are discussed in Section 4.12.
4.4 Deposition and region of the lower respiratory tract

As observed above (Table 4-2) and as supported by further MPPD calculations with other particle size distributions, deposition fraction in humans or rodents and $D_F/T_D$ ratios can differ considerably in the various regions of the lower respiratory tract (LRT). There is currently no clear guideline to handle this uncertainty:

- For some respiratory effects, only deposition in the pulmonary region (PU) is relevant, for others the (lower) TB region should also be considered. The critical target cells are not always known, and more than one mode of action may be involved.
- For the same substance, different effects (e.g. carcinogenicity and COPD or inflammation) may occur at different sites within the LRT, but only one HEC is calculated.
- The most relevant site may differ between species, the PU region for the one species and the TB region could be more important for the other species (see also Section 4.5, below).
- Even though the absolute deposited dose in the one or other region may be low and does not considerably contribute to the overall particle load in the respiratory tract, this fraction may be decisive and may greatly differ between species, leading to changes in $D_F/T_D$ ratios.
- Allocated deposition sites should fit to the subsequent steps for HEC calculations, i.e. normalisation and clearance. Therefore, the unit for normalisation (lung surface or lung plus TB surface or volume of macrophages etc.) should be selected in accordance with the critical deposition site. Clearance mechanisms will be different for particles deposited in the TB or in the PU region and more than one elimination pathway may be relevant and depend on the primary deposition site or the secondary site within the LRT.
- Subsequent calculations for retention and elimination adopt the original deposition fraction as a starting point. But this may not be correct after redistribution and translocation, where the fraction deposited in the TB or the PU region, respectively, may have changed with different relative body burdens.
- Specifically for larger particles (i.e. particle size > 2 µm), species differences increase: deposition in TB may be more important in rodents for the coarse particles and less relevant for humans and restriction to the pulmonary area may, thus, not be justified.

Currently, the default procedure in the German HEC calculations for deposition by MPPD includes the PU plus the TB region. This is discrepant from the respective assumptions used for normalisation and for clearance and, therefore, implies some uncertainties, which become more evident with the more recent MPPD 3.04 version (see example in Section 4.3.3). A more accurate region to refer to would probably be the PU plus the lower TB area. Specifically, for some tumours of the respiratory tract the region of concern may not include the upper TB region including trachea, but again, there are significant substance-specific differences and in most cases critical regions for deposition, translocation and final tissue interaction are not sufficiently known. To average deposition fraction over all potential interaction sites may thus lead to
unjustified species differences, and the HEC does not reflect the true (decisive) differences.

4.5 **Inhomogeneous deposition**

Selecting the adequate tissue or site in the LRT for calculating deposition is a key question. However, it is not limited to selecting the PU or TB tract as a whole, but may need to be extended to critical spots within the PU or TB area, which may be crucial for adverse effects, whereas other areas are less relevant.

We have already raised this issue in an earlier discussion on HEC (FoBiG, 2011), where we cited studies concluding that locally accumulated concentrations, e.g. at bifurcations of the respiratory tract, are more relevant than the overall average deposition level of particles within the LRT or PU region. Inhomogeneity of deposition can be expressed as “hot spot deposition enhancement factors” (DEF) with highly elevated deposition by 1 or 2 orders of magnitude. For example, Phalen et al. (2010) reported such DEFs >100. More recent documentations provide convincing evidence for such inhomogeneity, also demonstrating that the magnitude of those disparities depends on particle size, which makes it even more complicated to find an adequate DF_{P}/DF_{H} ratio for an appropriate local region of the respiratory tract (Dong et al., 2019).

Balasházy et al. (2003) also reported high enhancement factors at hot spot areas and concluded: “Early histological studies already indicate that neoplastic and preneoplastic regions predominate at bifurcation regions of the central airways”. Therefore, the hot spot concentrations in deposition may be crucial for subsequent effects. This is also supported by a recent study by Füri et al. (2020), who found serious inhomogeneity of radon deposition in the human lung: According to the authors, “the study demonstrates that the cell nuclei receiving high doses are non-uniformly distributed within the bronchial airway generations. The results revealed that the maximum of the radiation burden is at the first few bronchial airway generations of the respiratory tract, where most of the lung carcinomas of former uranium miners were found.”

It was suggested to calculate deposition masses in close vicinity to the respective hot spots for HEC calculations. For example, Donaldson et al. (2008) proposed to use the “proximal alveolar regions” (PAR) for deposition normalisation (Section 5.4 and Section 6.7). However, those approaches have not yet been adopted in regulatory risk assessment guidelines.

4.6 **Deposition and solubility**

Currently, MPPD -deposition modelling in the lower respiratory tract of particles does not consider solubility. However, for water-soluble particles hygroscopic properties can influence deposition patterns (Varghese and Gangamma, 2009). For example, water-soluble metal salts like cobalt chloride or zinc sulphate increase in size (“hygroscopic growth”), when entering the respiratory tract (Ferron et al., 2013). For sodium chloride with a dry diameter of $\approx 1 \mu m$, a growth factor of 6 has been reported (Ferron et al., 2013). Winkler–Heil et al. (2014) described more specifically: “due to the variability and
asymmetry of the human airway system, individual trajectories of inhaled particles are associated with *individual* growth factors, thereby enhancing the variability of the deposition patterns." For example, the authors described individual growth factors between 1 and 3.5 for particles with an initial dry size of 3 µm. Moreover, there are species differences in particle hygroscopic growth due to the different amount of time a particle travels through the regions with high relative humidity. The flow regime in the rat upper airways influences total and regional deposition much less than it does in human airways (Ferron et al., 2013). Because the turning points of the deposition probabilities differ between species, no linear relationship between hygroscopicity and the $DF_\text{T}/DF_\text{H}$ factor can be established.

Therefore, hygroscopic growth leads to significant uncertainty and variability in HEC calculations for water-soluble particles. These uncertainties are currently not addressed in the HEC procedure and not covered in MPPD deposition calculations. Asgharian et al. (2014) explicitly confirms with respect to MPPD 3.04: "consideration was not given to the potential for differences between species of hygroscopic growth of particles, which could influence predictions of the respirable fraction."

### 4.7 Deposition and density

In Germany, the OEL for PSLT particles has been derived from animal data and HEC has been applied for interspecies extrapolation. This OEL is derived for a PSLT particle with standard density of 1 g/cm³ (and needs to be adapted for densities deviating from 1 g/cm³ by simply multiplying with the substance specific density). Therefore we were interested to know about the influence of density on deposition in experimental animals and humans (influence of density will also be analysed within "dose metrics and normalisation"; see Section 5).

From a modelling approach by Braakhuis et al. (2014) there are indications that density at identical particle sizes significantly influences deposition. However, Morfeld et al. (2015) questioned such a significant impact. Therefore, we analysed the influence of density and MMAD for a broad range of densities, using MPPD version 3.04 (Table 4-4).
Table 4-4: Influence of density changes (0.1; 1; 5 g/cm³) in combination with particle size (MMAD: 0.5, 2, 3, 4 µm; GSD: 2) on deposition fractions and DF_T/DF_H. MPPD 3.04; body weight Sprague-Dawley rat: 370 grams (default allometric breathing volume); 1 mg/m³

<table>
<thead>
<tr>
<th>MMAD [µm]</th>
<th>GSD</th>
<th>Density [g/cm³]</th>
<th>DF_T</th>
<th>DF_H</th>
<th>DF_T/DF_H</th>
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</thead>
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<td>0.0478</td>
<td>0.0511</td>
<td>0.94</td>
</tr>
<tr>
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<td>1</td>
<td>0.0869</td>
<td>0.0827</td>
<td>1.05</td>
</tr>
<tr>
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</tr>
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<td>0.0781</td>
<td>0.31</td>
</tr>
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<td>5</td>
<td>0.0135</td>
<td>0.0697</td>
<td>0.19</td>
</tr>
</tbody>
</table>

From this analysis we conclude:

- For small particles (MMAD ≈ 0.5 µm), depositions in rats and humans are similar, leading to DF_T/DF_H ratios close to 1 (i.e. 0.94 - 1.05) with only minor impact of the different densities.
- For particles with MMAD within the applicability domain of the HEC default procedure (Section 2.2), the influence of density on the deposition fraction ratio is limited, with an increase of DF_T/DF_H ratio by a maximum factor of 1.42 (for densities 0.1 – 5 g/cm³) or less for the examples calculated.
- For particle diameter > 3 µm, there is a larger influence of density on deposition in rats than in humans. At a density of 0.1, only a small fraction is predicted to be deposited in the rat lung (0.1%), whereas deposition is significantly higher in humans for such particles (i.e. > 6%).
- Generally, the influence of density on deposition is larger in the rat compared to humans.
These observations indicate that the impact of density on deposition is only small within the applicability domain of the default HEC procedure and is not linearly correlated with HEC. Therefore, the current handling of density within the PSLT OEL concept in Germany (normalisation of the OEL to a density of 1) is not justifiable by relative deposition, but, possibly, by the normalisation of dose with density (see Section 5.7 for further discussion).

4.8 Deposition and particle size

In Section 2.2 we defined an applicability range for default HEC calculations by particle diameters from 0.5 to 2 µm. This is suggested because of some substantial uncertainties for HEC calculations outside this range:

- Kuempel et al. (2015) describe different deposition mechanisms below 0.5 µm, with diffusion dominating at smaller sizes. The rapid increase of the deposition fraction at smaller particles sizes contribute to the overall uncertainty. The authors state: “The deposition of inhaled substances in the human respiratory tract depends on the aerodynamic diameter for particles larger than approximately 300–500 nm in diameter or on the diffusion diameter and density for smaller particles (including nanoparticles) …. The main deposition mechanisms are impaction, sedimentation, and interception for particles with aerodynamic diameters greater than approximately 500 nm, whereas diffusion is the predominant deposition mechanism for smaller particles…. These competing deposition mechanisms result in minimal deposition efficiency at approximately 500 nm”.

- Gregoratto (2010) states that translocation of particles to the lung interstitium is specifically relevant at particle sizes below 0.5 µm in humans and less relevant above. Therefore, default HEC application is specifically uncertain at this low end of microsized particles.

- OECD (2018) set a quality standard from animal inhalation studies with 2 µm MMAD as maximum particle diameter of exposure.

- Calculation results with MPPD version 3.04 predicts that deposition of particles > 3 µm in rats is minimal leading to extremely uncertain and high DFT/DFH ratios (Table 4-4).

- As indicated by calculations by ARA (the editors of MPPD 3.04) (Asgharian, 2018; 2019), variability in individual deposition increases substantially in the range of very small (< 0.1 µm) or rather large (> 2 µm) respirable particles (Figure 4-5).

This limited size range for default extrapolations does not preclude case-by-case decisions to calculate HEC in the more uncertain range of particle sizes outside.

Interspecies comparisons on deposition of particles in the respiratory tract are clearly influenced by the particle size selected in the experimental animal study: if the animal (rat) study has used very fine particles (e.g. 0.1 µm MMAD), the DFT/DFH ratio is close to 1 (no substantial quantitative interspecies differences in deposition). If, however, rats were exposed to more coarse particles (e.g. 3 µm MMAD), HEC will be much lower.
than the respective concentration in the animal study (i.e. DFₜ/DFₜ < 1). Table 4-5 and Table 4-6 and corresponding Figure 4-1 and Figure 4-3, respectively, demonstrate the relationship between MMAD and deposition for a broad range of particle sizes for rats and humans and two sets of data with different densities of particles and different rats strains (different rat body weight); Figure 4-2 demonstrates the corresponding changes in DFₜ/DFₜ for the data from Table 4-5.

Results shown in Figure 4-1 and Figure 4-3 were somewhat unexpected. All similar presentations (e.g., Greim, 1997, Figure 4-3) of the deposition fraction in the pulmonary region for particle sizes above 1 µm MMAD showed a local maximum of deposition in rats at 2 or 3 µm diameter. This has also been demonstrated with using earlier versions of MPPD (2.01 or 2.11), as shown in Figure 4-4. No such local peak in deposition fraction has been found with the data from Table 4-5 and Table 4-6.

The specific background of this apparent change in deposition modelling in MPPD 3.04 has not been discussed in published comments.

Note that the particle size selected in the experimental animal study is regarded representative for all sizes of respirable particles; however, exposure to humans may be to larger or smaller respirable particles than those in the experimental study. Therefore the selection of particle sizes in the animal study will lead to more or less conservative OELs, depending on the particle size profiles of respirable aerosols at the workplace compared to the particle size profile in the “point of departure” animal study.

The effect of particle size on deposition should also be considered, when discussing the impact of particle growth for water-soluble particles on deposition (see Section 4.6): the resulting DFₜ/DFₜ ratio might be different for larger particles compared to the original (“dry”) particle size fraction. Therefore, it is hardly possible to make general predictions about quality and quantity of changes to the DFₜ/DFₜ ratio valid all over the range of respirable particle sizes.
Table 4-5: DF\textsubscript{T} and DF\textsubscript{H} and DF\textsubscript{T}/DF\textsubscript{H} ratios for a broad range from MMAD (rat: 0.1-3 µm; human: 0.1-5 µm) (Input data: rat body weight 400g; Sprague-Dawley; default assumptions on breathing volume, FRC and URT; default assumption for workplace scenario; particle density 4.0; GSD: 1.8; exposure conc. 1 mg/m\textsuperscript{3}); PU-region and PU+TB-region

<table>
<thead>
<tr>
<th>MMAD [µm]</th>
<th>RAT (DF)</th>
<th>Hum (DF)</th>
<th>PU</th>
<th>PU+TB</th>
<th>DF(T)/DF(H)</th>
<th>DF(T)/DF(H)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PU</td>
<td>PU+TB</td>
<td>PU</td>
<td>PU+TB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
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Figure 4-1: Particle Size and deposition in rat and humans from MPPD (3.04) calculations, density 4 (Input data: Table 4-5)
Table 4-6: DFₜ and DFₜ/DFₜ ratios for a broad range from MMAD (rat: 0.1-3.3 µm; human: 0.1-5 µm) (Input data: rat body weight default; asymmetric Long-Evans; default assumptions on breathing volume, FRC and URT; default assumption for workplace scenario; particle density 1.0; GSD: 1.5; exposure conc. 1 mg/m³), PU region only

<table>
<thead>
<tr>
<th>MMAD[µm]</th>
<th>RAT</th>
<th>Hum</th>
<th>PU</th>
</tr>
</thead>
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<tr>
<td></td>
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<td>DF(H)-PU</td>
<td>DF(T)/DF(H)</td>
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</tr>
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Figure 4-2: DFₜ/DFₜ ratios as a function of MMAD for the example data calculated in Figure 4-1, density 4 (Input data: Table 4-5)
Figure 4-3: Particle Size and deposition in rat (DF\textsubscript{R}) and humans (DF\textsubscript{H}) from MPPD (3.04) calculations, density 1. (Input data: Table 4-6)

Figure 4-4: Deposition fraction in rats versus particle size according to MPPD version 2.11 or 2.01, resp.; both lines are non-monotonous with a second local maximum > 1 µm; exper. Data are from Raabe et al. (1988; 1976) (figure unpublished, sourced from internal discussions in German OEL-setting committee; d is aerodynamic equivalent diameter; discussed in Section 4.3.3).
4.9 Inhalability adjustment, applying MPPD

When applying MPPD 3.04 (or earlier versions) to calculate deposition the user can optionally tick “inhalability adjustment” or do calculations without inhalability adjustment. If calculations are performed without “inhalability adjustment” the inhalability fraction is set to 1.0, i.e. all particles are regarded as inhalable. If “inhalability adjustment” is ticked, the inhalable fraction is reduced, specifically in experimental animals, but only to a negligible degree in humans. If no “inhalability adjustment” is applied, the absolute and the fractional amount deposited in the respiratory tract of experimental animals is overestimated. Therefore omission of the “inhalability adjustment” has been criticized for certain applications of the MPPD modelling (Morfeld et al., 2015). However, the online-guidance to MPPD 3.04 does not generally request application of this inhalability adjustment: “this adjustment is relevant for particle sizes larger than 3-4 microns for rats and larger than about 8 microns for humans; the probability that particles larger than these are inhaled is less than 1.0 and decreases with increasing particles size…."

In conclusion, there will be some difference in deposition calculations, if inhalability adjustment is ticked or not. However, the difference is rather small in the default region for HEC calculations (Section 2.2). If, however, MPPD is used beyond that range, consequences will be significant. We suggest applying inhalability adjustment in any case, because this avoids underestimation of effects, even though the consequences will be marginal in the default region of applicability.

4.10 Deposition variability

The data presented above (Sections 4.3 to 4.8) demonstrate some uncertainty and variability in deposition calculations as part of the HEC procedure. Breathing volume and activity changes in experimental animals and humans, airflow modelling uncertainties, regional and local inhomogeneity in deposition patterns, influences of water-solubility on particle growth, influences of density and particle size, all contribute to a rather broad range of values for DFₜ and DFₜH fractions and consequently for the DFₜ/DFₜH ratio. Uncertainties may not be fully discriminated from variability. Figure 4-5 provides an example for variability in the regional deposition fraction (intraspecies variability for different particle sizes and for PU vs. TB region). The author comments on the background for this variability: “explanation is that variation increases when external forces increase: diffusion for ultrafine, and impaction and sedimentation for coarse particles. These effects cancel each other out and deposition and also variation is reduced in the sub micrometer range.” (B. Asgharian; personal communication; April 8th, 2020). Such significant variability at the higher and lower range of particle sizes led to limiting the applicability range of the default HEC procedure, because selection of the most appropriate DFₜ/DFₜH ratio includes increased uncertainties (Section 4.4).
Furthermore, differences in deposition due to compromised health of exposed persons at the workplace may contribute to overall variability. Generally, for OEL assessment scenarios, it is assumed that persons with moderately impaired respiratory health are frequently still attending daily work. Some respective comments are listed below:

- „Lung deposition may be altered in various pathological states, such as bronchitis, emphysema and fibrosis“ (Bos et al., 2019)
- “It is found that the PD [particle deposition] in models with… COPD has been disrupted by the geometrical changes and followed airflow alternations. …For COPD, the stenosis location determines the effects on DE [deposition efficiency] and DF [deposition fraction]. … DE increases with the particle size, and DE of the terminal bronchi is higher than that of central regions.” (Zhang et al., 2018)
- “…These predefined parameters [e.g. in MPPD] do not include, for example, airway diameters and alveolar volume. …. This significantly limits the usefulness of these in silico lung models when moving from the healthy to the COPD lung.” (Ganguly et al., 2019)
- “Uncertainties in the deposition of nanoparticles in the lung will remain due to considerable intersubject variability in lung morphology, breathing pattern, and
possibly even circadian rhythms affecting the respiratory tract. This is particularly relevant for vulnerable subgroups of the population.” (Löndahl et al., 2014).

Variability in deposition is not covered by the HEC value and adds to the uncertainty of the HEC procedure.

4.11 MPPD deposition for mice

MPPD version 3.04 permits to calculate deposition rates for mice. Parameters were derived from BALB/c and B6C3F1 mice. The calculation procedure is largely identical to the procedure documented for rats (Section 4.3.2). However, it should be noted that the current template may contain an erroneous default value for the tidal volume of mice. We suggest to enter a default breathing frequency of 296 breaths per minute and a default tidal volume of 0.229 mL for a 30 g mouse in the exposure template of MPPD. With these data for mice and the particle characteristics also used for illustration in Section 4.3.3, Table 4-2, we calculate example DFT/DFH ratio as shown in Table 4-7. In this example, deposition fraction in the PU-region of mice is higher than in rats and deposition fraction in the TB-region is higher than in humans (and in rats). However, this relatively high deposition in the mouse TB region may be specific for only a small particle size range. Kolanjiyil et al. (2019) provide a general statement: “for micron-particles, the tracheobronchial deposition and alveolar deposition are significantly higher in the human lung than that in the mouse”. The authors also note: “the submicron deposition in the human distal lung airways is consistently lower than that in mouse-airway generations”. Mice data should not be used for species extrapolation at particle sizes above 3 µm. Similarly, Asgharian et al. (2014) give a warning: “there was little or no deposition of 3 µm and larger particles in the LRT [of mice]”. In a general statement, Kolanjiyil et al. (2019) acknowledge that only limited experimental data are available in the literature on mouse lung deposition. An analysis, comparing the deposition fraction from acute exposure of particles in the mouse lung from MPPD (version 3.0) and Raabe et al. (1988) by Ali et al. (2017) indicated major differences in deposition fractions in the two references. For example, MPPD calculated a PU deposition fraction of 12.37% for particles with MMAD of 190 nm, whereas the Raabe et al. in vivo data indicated a PU deposition fraction of 45.4%. For a larger particle (MMAD: 767 nm) the MPPD calculation was 2.73% (PU deposition), where Raabe et al. found 9.7%.
Table 4-7: MPPD calculations (version 3.04) for mice; input data: MMAD: 1.4 µm; GSD: 2.1; Density: 2.0 g/cm³; concentration: 0.067 mg/m³, body weight 30 grams mice, breathing frequency: 296; Tidal volume as from allometric formula (see Section 3.4)

<table>
<thead>
<tr>
<th></th>
<th>MPPD 3.04</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depos.</td>
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</tr>
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</tr>
<tr>
<td>Mice</td>
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</tr>
<tr>
<td>DF&lt;T/DF&gt;H</td>
<td>0.608</td>
</tr>
</tbody>
</table>

4.12 Summary and conclusions on deposition

Deposition calculation provides important input for HEC interspecies extrapolation. However, several limitations, uncertainties and variabilities have to be acknowledged:

- In the default procedure, the particle size range is limited to MMADs of 0.5 - 2 µm, because
  a) at the low diameter end (< 0.5 µm), other deposition principles become predominant,
  b) at the low diameter end (< 0.1 - 0.5 µm), deposition fractions are highly influenced by the steep slope from maximum to local minimum deposition over an extremely small difference of size,
  c) below the lower diameter end, for the nanosized particles (< 0.1 µm) the deposition calculations are reported to be highly uncertain,
  d) at the high end of diameters, OECD guidelines request to limit diameters of test materials in animal studies to 2 µm,
  e) at the high end of diameters (> 3 µm) pulmonary deposition in experimental animals will be very low and may lead to overly conservative DF<T/DF>H ratios, and
  f) interindividual and intraindividual differences in deposition fractions due to breathing patterns and airway anatomy will rapidly increase beyond the default range of diameters in humans and therefore contribute to additional uncertainty of the DF<T/DF>H ratio.

- However, case-by-case calculations of DF<T/DF>H ratios with adapted parameters beyond the default HEC calculation range can be considered.
- The deposition modelling by MPPD does not include hygroscopic growth, which, however, is considered relevant, e.g. for water-soluble substances, as is demonstrated by significant changes of DF<T/DF>H, in response to minor particle diameters changes.
- Calculations with the MPPD 3.04 software disclose the significant influence of body weight (and, therefore, breathing frequency and tidal volume) on overall daily breathing volume in rats and, subsequently, on DF<T/DF>H. With the earlier
MPPD 2.11 it was not directly possible to take account of those strain-specific parameters.

- Calculations with MPPD demonstrate a decrease of deposition fraction at higher particle diameters in experimental animals in the pulmonary region, which is non-monotonous with a local maximum at 2 or 3 µm (MPPD 2.11, MPPD 2.01) and which is monotonous (no such local maximum) for MPPD 3.04. The background for this difference is unknown.
- Additional rules need to be developed: should deposition in the pulmonary region be addressed only, or should the tracheobronchial region (or parts of the TB-region) be included additionally.
- Moreover, local deposition at “hot spots” (e.g. bifurcations of the airways) may lead to highly inhomogeneous distributions, and average deposition in the lung or TB-region may be meaningless compared to such hot spot enhanced deposition sites. There are differences between species and mechanisms of such local depositions are not linearly correlated with average deposition. There are indications that (at least, some) neoplastic and non-neoplastic effects occur at such hot spots as target site.
- Breathing patterns, due to, e.g., nose or mouth breathing or exercise and influenced by physiological parameters, demonstrate high variability (human inter-individual differences) for submicron-sized and for large respirable particles. Impairment of respiratory health by particles will alter deposition fractions significantly, as, for example, has been shown for COPD.
- From the limited data available for validation of the HEC calculations based on mice data and from the uncertainty analysis by Kolanjiyil et al. (2019), we conclude that interspecies particle deposition estimates based on mice data are associated with substantial uncertainty.
5 Normalising Factor (NFH / NFT) and Dose Metrics

5.1 Normalisation and dose metrics – overview

Even after adjusting for species differences in weighted breathing volume (Section 3) and deposition (Section 4) the air concentration with unit mg/m³ might not be the best measure to compare potencies of different particles in the respiratory tract. Exposure needs to be quantified as a dose (measured in appropriate dose metrics) and needs to be related to a meaningful reference unit in the target organ. These steps are accomplished by assigning dose metrics to the deposited particles and by normalisation. For HEC calculation, the interspecies ratio of the dose after normalisation are of interest, i.e. the ratio of normalising factors (NFH/NFT).

Note that in the current formula for HEC (Section 2.1), NFH/NFT does not include dose metrics explicitly and the ratio of normalisation factors calculated for a specific particle would be identical regardless of the dose metrics applied.

However,
- selection of specific dose metrics may be more or less appropriate for the various potential modes of action,
- selection of specific normalisation may be more or less appropriate for the various potential modes of action,
- the final HEC provided, e.g., as mass concentration (mg/m³) is different from the final HEC provided, e.g., as volume concentration (mL/m³) as dose metric,
- there are substance-specific differences in transformation of HECs provided as, e.g., volume concentration into, e.g., mass concentration,
- HECs for several particles can only be compared adequately, if provided in identical dose metrics.

Because similar considerations with respect to the mode of action are necessary for selecting dose metrics and normalisation, we discuss both steps of HEC quantification in this Section 5, even though transformation of HEC into the adequate dose metrics could also be analysed within the last step, the aggregated HEC calculation (Section 7).

Even though it would be helpful to generate results with several dose metrics and normalisations for one set of exposure data for discussion of possible modes of action (MoA), such complete data are rarely available and some have to be approximated, if needed. Moreover, regulatory purposes require a final output as mg/m³ (e.g. as an OEL, with mass concentration as final metric). Therefore, some suboptimal dose metrics may be considered to be acceptable considering easy calculations and easy transformation into pragmatic regulatory values.

The most serious problem in providing appropriate dose metrics and normalisation procedures is their dependency on a specific MoA and the typically large uncertainty
about this MoA. If, for example, the impairment of alveolar macrophage function is the key mode of action, the appropriate normalisation may be the volume of alveolar macrophages, and the volume of the particles would probably be the adequate dose metric. But for many types of particles, the MoA is not unambiguously known and/or more than one MoA may be relevant.

Solubility of the particle may greatly influence the MoA in the respiratory tract (with respect to, e.g. primary target tissue, intracellular uptake, binding to proteins). In the discussion below, with solubility we refer to solubility in physiological lung fluids and not primarily to solubility in water. Therefore, suitable dose metrics and normalisation may have to be differentiated for the different solubility in lung fluids and different related MoAs. Further, if the average deposition in the respiratory tract is not determining the effect, but instead local deposition at hot spots is critical, this should be addressed by specific normalisation units. And if effect-related deposition (or translocation) is relevant also in the TB area, this calls for to a different normalisation compared to just the PU region reference.

We address dose metrics and normalisation separately and stepwise. This includes
- discussion of the German approach for an PSLT OEL with respect to dose metrics and normalisation (Section 5.2),
- discussion of various dose metrics and their suitability for different types of MoAs (Section 5.3),
- discussion of advantages and disadvantages of different normalisation units and ways to quantify them (Section 5.4),
- impact of solubility of particles on normalisation (Section 5.5),
- specific aspects on normalisation for HEC based on mice data (Section 5.6), and
- summary and conclusions (Section 5.7).

### 5.2 The German PSLT-approach and dose metrics/normalisation

In 2014, in Germany an OEL for PSLT-particles was established, at

1.25 mg/m³ (respirable) PSLT particles for dust density of 2.5 g/cm³, i.e.,
0.5 mg/m³ (respirable) PSLT particles for dust density of 1 g/cm³ (AGS, 2014).

This regulatory OEL is only slightly different from the corresponding “MAK-Wert” by DFG (2019) of 0.3 mg/m³ (respirable) PSLT particles for a dust density of 1 g/cm³. Both values are based on a background paper, coedited by DFG and AGS authors (Hartwig, 2012). This assessment was based on two approaches with similar results:

- **Approach A**: based on two animal studies (with exposure to toner particles and titanium dioxide particles) animal NOAECs were reported and HEC was calculated by the formula documented in Section 2.1 of this report. Dose metric was mass (mg) and normalisation was done by referring to the lung surface area. HECs were originally calculated as mass concentration in air (mg/m³). Subsequently, in a separate step, the
results were transformed to a density of 1 (given density of toner dust was 1.2 and of titanium dioxide 4.3, respectively):

- The HEC for toner of 0.13 mg/m³ at density 1.2 was converted to a concentration of 0.11 mg/m³ for unit density.
- For titanium dioxide the original HEC of 1.1 mg/m³ resulted in 0.25 mg/m³ after transformation to density 1.

For justification, the authors explain: “Even if - in case of approach A - the deposited dose per square meter lung surface is calculated, for chronic exposure one needs to take account of the retained particle dose. The retained dose depends on the particle clearance. Particle clearance is influenced by the particle density / particle volume. Therefore density needs to be considered for approach A.” (non-literal analogous translation from Hartwig, 2012)

**Approach B:** the approach was based on an analysis by Pauluhn (2011b) and postulated a MoA, under which the threshold for effects of PSLT would be linked to a particle volume of 6% of the total volume of alveolar macrophages, described as “overload-threshold” for inert particles by the authors. The threshold was calculated to be a volume-based generic mass concentration of 0.5 µl respirable particles/m³ × density. This results in a threshold of 0.5 mg/m³ for particles with a density of 1 in humans. Data of various PSLT particles including those for titanium dioxide (0.5 mg/m³ corresponding to 2.15 mg/m³ for a density of 4.3) fitted quite well to this postulated generic quantification. The result was also supported by data for nano-particle-agglomerates, if agglomerate density is used for dose metrics.

Approach B with dose metrics of particle volume and normalisation to the macrophage volume was applied in later German HEC calculations for nano-PSLT (AGS, 2015). With respect to normalisation and dose metrics, we conclude that normalisation to **alveolar macrophage volume** and dose quantified as **particle volume** are the factual current default procedure of HEC calculations for PSLT particles in Germany, although the official default for PSLT and other particles in the generic HEC guidance still is mass for dose metrics and “alveolar plus tracheobronchial surface area” (Oberdörster, 2010) for normalisation (AGS, 2013).

### 5.3 Alternatives in dose metrics

MPPD, version 3.04, provides only **particle mass** related information as dose metrics of their output for subsequent calculations, i.e.

- Deposition fraction (mass deposited/ inhaled)
- Deposited mass (mg)
- Deposited mass rate (mg/min.)
- Deposited mass per surface area (mg/cm²)
- Deposited mass flux (mg/min/cm²).

Transformation to other dose metrics may be possible but are not included in MPPD 3.04. For example, number of particles or particle surface or particle volume are also
frequently discussed as potential dose metrics. However, MPPD 3.04 also considers particle volume implicitly, as far as deposition calculations combine diameter (MMAD) and density for their calculations.

5.4 Alternatives in normalisation

As indicated above, the adequate unit for normalisation depends on the mode of action (MoA). Some dose metrics (Section 5.3) are closely correlated to normalisation. For example, particle volume is linked to the alveolar macrophages volume for normalisation to be meaningful, if macrophage particle loading is determining subsequent respiratory effects.

Generally, various alternative normalisation units are proposed, especially,

- Alveolar surface area or alveolar surface plus TB-area surface (m²)
- Lung weight (grams or kg)
- Lung volume (m³)
- Alveolar macrophages, number (n)
- Alveolar macrophages, volume (m³)
- Ventilatory units, number (n)
- Number of cells per lung (n)
- Surface area, type II epithelia cells (m²)
- Surface area, type I epithelia cells (m²)
- Proximal alveolar regions (PAR; m²) for hot spot correlation (Donaldson et al., 2008).

Many of those normalisation units are not regarded suitable for default calculations but can be considered case-by-case. The most frequently discussed units are i) Alveolar surface area or alveolar surface plus TB-area surface, ii) volume of alveolar macrophages, and iii) lung weight.

If those units were used for normalisation, numbers have to be selected to calculate \( \text{NF}_{\text{H}}/\text{NF}_{\text{T}} \). Therefore, the current quantitative values are discussed below:

Lung surface area

There is no general rule whether the alveolar surface or the surface of the PU plus TB region should be used, if surface area is regarded the most appropriate unit for normalisation. In Germany, \( \text{NF}_{\text{H}}/\text{NF}_{\text{T}} = 150 \) was used for normalisation (default according to guidance; AGS, 2013), which apparently has been chosen from PU plus TB region surfaces of rats and humans (Oberdörster, 2010). Other measures and fractions are listed below (Table 5-1).

The difference between the minimum and the maximum is 140 vs. 349, indicating differences up to a factor of 2.5. Lung surface for humans has been discussed controversially (Bruch, 2013; Gehr et al., 1978; Morfeld et al., 2015). Fröhlich et al. (2016) commented on the large values for human alveolar surface: “True alveolar surface available for gas is 20–50% smaller than the epithelial surface, depending on the level of air space inflation. At full inflation of 140 m², for instance, the “true” alveolar
surface is only 70–100 m². But still there apparently is no general agreement, as Morfeld et al. find that the large surface calculated by Gehr et al. in fact is not the epithelial surface but is the surface available for gas exchange.

Qualitatively, Oller and Oberdoerster (2010) suggest to select alveolar surface for normalisation: “The surface area normalized dose appears to be most useful for directly comparing doses in the respiratory tract between different animal species with vastly differing body sizes, like rat and human, considering that most effects are initiated by interaction of deposited particles with the epithelial cells of the respiratory tract and macrophages moving on the epithelial surfaces.” This concept is largely supported by Brown et al. (2005), stating: “If epithelial cells are the target, the TB- or alveolar surface area would be the most likely normalising parameter”. However, when the authors did not look to the target, but to the cause they suggested other normalising parameters for certain types of particles (below).

Table 5-1: Alveolar surface or PU- plus TB-surface in rats and in humans and NFH/NFT from different sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Humans [cm²]</th>
<th>Rat [cm²]</th>
<th>Ratio (NFH/NFT)</th>
<th>Remarks</th>
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</thead>
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<tr>
<td>(Hartwig, 2012)</td>
<td>567780</td>
<td>2950</td>
<td>192</td>
<td>data from SD-Rats</td>
</tr>
<tr>
<td>MPPD 2.11 listed values</td>
<td>572220</td>
<td>2970</td>
<td>191</td>
<td>according to Fröhlich et al., (2016), referring to EPA (2004)</td>
</tr>
<tr>
<td>Oberdoerster (2010)</td>
<td>630200</td>
<td>4125</td>
<td>153</td>
<td>rat strain not identified in source, includes PU+TB area</td>
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<tr>
<td>EPA (2008)</td>
<td>540000</td>
<td>3400</td>
<td>159</td>
<td>no source or strain provided</td>
</tr>
<tr>
<td>Fröhlich et al., (2016)</td>
<td>700000</td>
<td>5000</td>
<td>140</td>
<td>Based on Lenfant et al. (2000): 1m²/kg BW in mammals allometric scale</td>
</tr>
<tr>
<td></td>
<td></td>
<td>780000</td>
<td></td>
<td>ICRP (Hum) according to Fröhlich et al. (2016), referring to Guha et al. (2014)</td>
</tr>
<tr>
<td>Morfeld et al. (2015)</td>
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<td>4100</td>
<td>349</td>
<td>Morfeld et al., referring to Gehr et al.(1978) and Stone (1992) as their sources</td>
</tr>
</tbody>
</table>

**Volume of alveolar macrophages (AM)**

AM volume is most frequently proposed for normalisation in HEC calculations for particles, but usually i) the analysis is restricted to PSLT particles and ii) the discussion
is focusing on high exposure effects, where particle clearance via AM is impaired. The assumption then is that no adverse effects need to be considered at lower AM volume loadings for PSLT particles. The AM volume for normalisation consequently fits the assumed MoA (higher volume loading leading to persistent inflammation). However, AM volume is also more generally regarded relevant, as all particles (either poorly or readily soluble) are also taken up by macrophages. For example, some soluble particles may bind to endogenous proteins, therefore become less soluble and are subsequently phagocytosed by AM, contributing to AM load.

If AM volume is regarded the appropriate scale for normalisation, there is still some uncertainty about the quantification, due to different quantitative figures provided in different studies (Table 5-2). In Germany, recent HEC calculations for PSLT particles (nano-PSLT) have been performed using AM volume (Section 5.2). Specifically, the normalising factor by Pauluhn (2011a) of $\approx 1100$ for NFH/NFT has been adopted. This estimate is based on AM volumes as reported by Krombach (1997), which presented combined data from several sources. The authors derived the number of macrophages per lung by a body weight related regression equation for rats and from Oberdoerster (1995) for humans. Comparison of the ratio of normalising factors in Table 5-2 indicates a large range of values with a minimum of 278 and a maximum of 1110 (factor $\approx 4$). This range demonstrates serious uncertainties about the most appropriate AM volume data to be used and generalized as a default, if AM volume is regarded to most appropriate parameter to normalise the deposited (or retained) dose.

**Lung weight**

Alternatively to lung surface or AM volume the lung weight has been proposed for normalisation (Brown et al., 2005). Beyond the parenchymal tissue the lung weight is influenced by the weight of the lung interstitium, which may also be the target of particle effects (Section 6.4). Kuempel et al. (2001) reported a lung weight of $\approx 1000$ grams for humans and 0.9 grams for the rat, resulting in NFh/NFt - ratio of $\approx 1000$. Similarly, Pott and Roller (2001) reported a human lung weight of 953 - 1200 grams and 0.9 - 1 gram for the rat (Wistar and Fischer), resulting in an identical normalisation factor. It should be noted that this NFh/NFt of 1000 is at the upper end of the normalisation factor ratios derived from either lung surface or AM volume and therefore is not a precautionary factor, even though it may be justified for some (not all) of the pulmonary effects from particles exposure.

**Other units for normalisation**

No further alternatives are discussed as “default normalisation units” here, as we found no applications of other normalisation units in practical particle OEL assessments. Some earlier suggestions and further data have been presented in former reviews (e.g., Brown et al., 2005; FoBiG, 2011; Jarabek, 1995; Kuempel et al., 2015).
Table 5-2: Alveolar macrophages volumes in rats and humans and NFH/NFT from different sources for quantification

<table>
<thead>
<tr>
<th>Source</th>
<th>humans (x10^6)§</th>
<th>Rat (x10^6)#</th>
<th>Ratio (NFH/NFT)</th>
</tr>
</thead>
<tbody>
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<td>Geiser, 2010 (F344-rats)*</td>
<td>1474 x 5990</td>
<td>639 x 29.1</td>
<td>310.23</td>
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<tr>
<td>Geiser, 2010 (SD-rats)*</td>
<td>1474 x 5990</td>
<td>1058 x 26.9</td>
<td>474.82</td>
</tr>
<tr>
<td>Miller, 2000 (SD-rats)</td>
<td>1474 x 5990</td>
<td>1161 x 26.9</td>
<td>278.4</td>
</tr>
<tr>
<td>Miller, 2000 (F344-rats)</td>
<td>1474 x 5990</td>
<td>882 x 29.1</td>
<td>344.01</td>
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<tr>
<td>Pauluhn, 2011 (no specified rat strain)*</td>
<td>4990 x 7000</td>
<td>1166 x 27</td>
<td>1109.52</td>
</tr>
<tr>
<td>Kuempel et al., 2001 (no specified rat strain)**</td>
<td>2500 x 7000</td>
<td>1000 x 26</td>
<td>673</td>
</tr>
</tbody>
</table>

*) cited from FoBiG (2011)  
**) see also for further data  
§) first term: Average AM volume [µm³]; second term: number of macrophages (x 10^6) /lung for a human with body weight of 70 kg  
#) first term: average AM volume [µm³]; second term: number of macrophages (x 10^6) /lung for a rat with body weight of 370 grams

5.5 Influencing factors: mode of action and solubility

Solubility and bioaccessibility of particles in the respiratory tract are important factors in HEC calculations. As already discussed in Section 4.6, water solubility influences deposition. However, water solubility is a rather poor indicator to describe solubility in physiological lung media like epithelial lining fluid or in the intracellular environment from lysosomal fluid. Solubility in physiological lung fluids will also have significant impact on the mode of action in the respiratory tract and therefore on the most adequate normalisation and dose metrics and on retention, clearance and elimination from the lung.

In the current German HEC default approach, solubility is only addressed by its impact on the elimination factor and only based on water solubility (Section 6.3). The influences of solubility on MoA, normalisation and dose metrics are not discussed.

Consequences of solubility on normalisation, dose metrics and retention and the link to MoA will be further discussed in Sections 6.6 and 6.9. In Section 7.4, we propose a discriminating scheme to address MoA, normalisation and elimination for substances with different solubilities by integral categories.
5.6 Mice specific normalisation

Only few data for airway parameters are available for mice suitable for normalisation. Table 5-3 provides some data to compare the alveolar surface area and total alveolar macrophage volume in humans and in mice. However, the significant variability of the reported ratios of normalising factors (NF_H/NF_T) is to be emphasised, which extends if other human data but the data by Kuempel et al. (2001, 2015) are selected for human reference.

Table 5-3: Normalisation and ratio of normalising factors (NF_H/NF_T) in mice

<table>
<thead>
<tr>
<th>Source</th>
<th>Airway parameter</th>
<th>unit</th>
<th>Ratio (NF_H/NF_T) *</th>
<th>Strain/ Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asgharian et al. (2014)</td>
<td>alveolar surface area (∑ generation 15-21)</td>
<td>397.07 cm²</td>
<td>2569</td>
<td>B6C3F1-mice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>491.59</td>
<td>2075</td>
<td>BALB/c-mice</td>
</tr>
<tr>
<td>Hsieh et al. (1999)</td>
<td>alveolar surface area</td>
<td>1410</td>
<td>723</td>
<td>Not specified</td>
</tr>
<tr>
<td>Stone et al. (1992)</td>
<td>lung surface area</td>
<td>500</td>
<td>2040</td>
<td>Not specified</td>
</tr>
<tr>
<td>Knust et al. (2009)</td>
<td>total alveolar surface area</td>
<td>82.2</td>
<td>12409</td>
<td>CL 57 B6 mice [range: 63.3 cm², 101 cm²]</td>
</tr>
<tr>
<td>Hsieh et al. (1999)</td>
<td>alveolar macrophages, total volume</td>
<td>1421 x 10⁶ µm³</td>
<td>12315</td>
<td>Not specified</td>
</tr>
<tr>
<td>Stone et al. (1992)</td>
<td></td>
<td>1430 x 10⁶</td>
<td>12238</td>
<td>Not specified (19.2 grams)</td>
</tr>
<tr>
<td>Stone et al. (1992)</td>
<td></td>
<td>1300 x 10⁶</td>
<td>13462</td>
<td>Not specified (two calculations in identical source)</td>
</tr>
</tbody>
</table>

*) assuming an alveolar surface area of 1,020,000 cm² (Table 5-1; Kuempel et al. (2015)) and a total volume of alveolar macrophages of 17,500,000 x 10⁶ µm³ (Table 5-2; Kuempel et al. (2001)) in humans

5.7 Summary and conclusions on normalisation and dose metrics

Adequate normalisation and dose metrics selection are key steps within the HEC interspecies calculation procedure. However, as the most appropriate normalisation is closely linked to the mode of action and as mode of action often is insufficiently known or as more than one mode of action is relevant, the selection of the one or other normalisation unit and NF_H/NF_T ratio may often be premature or, at least, highly uncertain.
Only for unambiguous PSLT substances, remaining uncertainties are sufficiently limited to agree on AM volume for normalisation in default assessments.

Specifically, the influence of particle solubility is currently not sufficiently analysed on a generic level with potential consequences on

i) biokinetics in the lung,
ii) interactions with either alveolar macrophages or epithelium cells or both,
iii) endogenous protein interaction,
iv) direct vs. indirect starting points in activating immunologic response including macrophage- and PMN-activation,
v) the relevance of intracellular vs. extracellular contributions to effects,
vi) consequences (duration, pathways and species differences) in translocation and elimination from the lung (see Section 6.4), and
vii) gradual differences based on the rate of dissolution in the one or other lung fluid.

Considering that many particles cannot be clearly identified as PSLT particles, the influence of the chemical reactivity from either the surface of the particle or the solubilized particle is highly important for adequate HEC calculations. Normalisation to, e.g. the alveolar surface or AM volume or lung weight will shift the NF\textsubscript{H}/NF\textsubscript{T} ratio (see “aggregate 3 ratios approach” in Section 7.4).
6 Retention and Elimination (ELR_{H} /ELR_{T})

6.1 Retention and elimination overview

The fourth step of the HEC calculation (after considering the weighted breathing volume, deposition, and normalisation) is the quantification of species differences in elimination rate (ELR_{H}/ELR_{T}). For a first order kinetic process, elimination rate is defined as ln 2/t_{1/2} (with t_{1/2} = elimination half-life). Clearance half-life and elimination half-life are identical terms. The MPPD software includes a module to consider elimination. However, this step in HEC calculations may also be excluded and accounted for separately. This exclusion from MPPD is justified as the software does not specifically address the impact of different solubilities on elimination rate and there are concerns that species-specific clearance information is not updated even for PSLT particles (for further discussion: Sections 6.4 and 6.6).

The retained dose in the lung is regarded relevant, as it is assumed that adverse effects are linked to the long-term lung burden. However, this may not always be the case: if, for example, a compound is retained in the lung, but is quiescent, i.e. not biologically active, during certain periods of time, this reference to the retained dose would be misleading. Furthermore, the assumed first order kinetics may not always be justified, and the assumption of a multi-phase elimination process be more adequate. We will not address systemic effects from soluble particles after clearance from the lung, but there is a potential that particles are translocated to the lung interstitium; thus, the particles are cleared from the alveolar region, but interstitial effects still need to be considered. PSLT particles are mainly eliminated via the mucociliary escalator: for this clearance mechanism species differences in elimination half-life are well-known. However, species differences are less evident for other clearance mechanisms and it is often assumed that there are no species differences for readily soluble particles. However, these two categories (soluble particles without species differences and poorly soluble particles with fixed species differences) are regarded to be an overly simplified grouping. Any fixed elimination rate is an approximation only, with significant variability, e.g. due to individual airway anatomy and breathing pattern differences, inhomogeneous local retention in the various regions of the lung and due to changes in clearance due to illnesses. Consequently, we will discuss several topics in this Section on retention and elimination:

- Type of clearance mechanisms (Section 6.2)
- Current handling of the elimination rate in regulatory approaches (Section 6.3)
- Translocation to the interstitium and consequences for interspecies elimination rates (Section 6.4)
- Species differences in case of impaired clearance of PSLT substances (Section 6.5)
- Solubility and elimination rate (Section 6.6)
- Variation and inhomogeneity of the elimination rate (Section 6.7)
- Elimination rate in mice and interspecies extrapolation (Section 6.8)
- Summary and conclusions on retention and elimination (Section 6.9)
6.2 Clearance mechanisms and species differences

Because species differences in elimination rates were primarily discussed for PSLT particles, most discussions circle around mucociliary clearance. However, elimination and retention refer to various potential clearance mechanisms:

- Dissolution
- Physical translocation (e.g., mucociliary clearance)
- Phagocytosis by macrophages
- Lymphatic drainage (Jarabek, 2016).

Usually, lymphatic drainage is combined with translocation to the interstitium to just one clearance pathway. However, this pathway should be subdivided and both steps should be separately addressed (Section 6.4).

As indicated, there are species differences in mucociliary clearance. However, quantitative species differences can also be expected for phagocytosis and translocation to the interstitium and to the draining hilar lymph-nodes (Nikula et al., 2001). Only for dissolution no species differences are known. As dissolution rarely is an isolated clearance mechanism, it cannot be generally concluded that there were no species differences for soluble particles (Section 6.6).

6.3 Current handling of elimination

There is no generic regulatory procedure for quantifying interspecies differences in elimination. In Germany, the following factors are used for calculating HECs. For PSLT particles, there are data for clearance rates in the rat without impaired clearance (often called “without overload”). This clearance (or elimination) half-life is provided with ≈ 60 days (AGS, 2013).

In humans, clearance half-lives of 400 days or more are reported in literature (e.g., Hartwig, 2012; Jarabek et al., 2005; Snipes, 1989) and 400 days are used as default in the German procedure (AGS, 2013). Clearance rate is calculated from elimination half-life by

\[
\text{Clearance rate (CL)} = - \ln (0.5) / \text{elimination half-life (AGS, 2013)}
\]

From this, species differences in clearance rates can be calculated:

\[
\text{Clearance}_{T} = - \ln (0.5)/60 \, \text{d} = 0.0116 \, \text{per day}
\]
\[
\text{Clearance}_{H} = - \ln (0.5)/400 \, \text{d} = 0.00173 \, \text{per day}
\]

with

\[
\frac{\text{CL}_H}{\text{CL}_T} = \frac{\text{ELR}_H}{\text{ELR}_T} = 0.00173/0.0116 = 0.15
\]

This ratio is used for poorly soluble particles at doses with no impaired clearance. There is no separate clearance interspecies factor for the “impaired clearance” situation. In the German guidance document on exposure-risk relationships it is explicitly stated: “in this case (i.e. at doses leading to overload situations) the identical factor is used as for unimpaired clearance” (Section 4.3 (3) in ERR-guidance; AGS, 2013). However, effects observed in animal studies in the impaired clearance dose range are regarded as inadequate point of departure for quantitative cancer risk assessment. It is further stated that this elimination factor is highly uncertain, if used for effect doses with impaired clearance. In addition, interspecies elimination rates ratio
(ELR_H/ELR_T) are not changed in the current German HEC procedure, if the particle is poorly soluble but exerts its effects by some chemical reactivity. Again, this constant ELR_H/ELR_T is characterised by AGS (2013) as being uncertain, if used for such chemically active substances.

For highly soluble particles, ELR_H/ELR_T is set to 1. No species differences in elimination are assumed in this case. For particles with an “intermediate solubility” the default factor for poorly soluble particles is doubled, i.e. it is set to 0.15 x 2 = 0.3. This factor of 0.3 has been established pragmatically, with no specific empirical background. Solubility is used identically to “water solubility”. However, there are no definitions for “soluble” or “intermediately soluble” provided. A case-by-case decision is suggested (for further discussion on the impact of solubility on the elimination factor, see Section 6.6).

Therefore, it should be acknowledged that the ELR_H/ELR_T ratio is rather uncertain for many types of particles. However, in the guidance document (AGS, 2013) the value of 0.15 is regarded to be conservative, which may be questioned based on the observations with respect to elimination from the lung to the interstitium (Section 6.4).

### 6.4 Translocation to the interstitium and consequences for interspecies elimination rates

In an earlier report (FoBiG, 2011), we have reported the data on human elimination half-lives for particles in some more detail. The default assumption of 400 days has been adopted from Hartwig (2012) derived from data. Other sources reported half-lives of about 30 days for 30% of the inhaled dose (phase 1 elimination) and of 700 days for 70% with a variability from 150 to 2500 days (phase 2 elimination; Snipes, 1989). It had been acknowledged that some of this retained fraction may have been translocated to the interstitium, specifically in monkeys and in humans (Nikula et al., 1997). As translocation to the interstitium was not regarded as retention but as elimination from the lung, the estimated elimination half-life of 400 days was still regarded as conservative. Morfeld et al. (2015) criticized this 400 days value and suggested a clearance half-life of 250-300 days or 230 days, based on data from Gregoratto et al. (2010) and estimated from allometric considerations. In fact, Gregoratto et al. derived a clearance half-life of 300 days in humans, however, this was restricted to the fraction, which is cleared to the ciliated airways via the mucociliary escalator. In addition, the authors found a significantly longer elimination half-life (about 40% of the lung deposit) for insoluble particles “sequestered in the interstitium”. For example, for radioactive 60Cobalt-particles the total elimination half-life increased to about 1924 days, which is similar to the upper-range figure reported by Snipes (1989). The crucial question was whether the translocation to the interstitium should be assumed to be retention in a critical tissue of the lung or whether the translocated dose is to be regarded as already eliminated from the critical zone. This is directly linked to the question, whether effects can also occur in the interstitial region of the lung. If translocation to the interstitium is considered a way of elimination, then the ELR_H/ELR_T – ratio decreases (larger interspecies differences, because ratio is < 1), if just mucociliary clearance is covered, ELR_H/ELR_T increases (less conservative). A potential highly significant difference in translocation to the interstitium in rats vs. humans is schematically shown in Table 6-1.
In more recent assessments, it was found that the amount translocated to the interstitium should not be regarded as eliminated (Gregoratto et al., 2011; Kuempel et al., 2001): specifically in humans it was observed that severe effects like fibrosis can be observed in the lung interstitium. Therefore, in the interstitial-sequestration model, Kuempel et al. (2001) included the interstitium, when calculating clearance half-time, whereas in earlier models (original HRTM-model and HRTM-modified model 1 from ICRP) this compartment was largely excluded (“Equivalent model”). Inclusion of the interstitium implies a more complex multi-phase elimination model instead of the simple first order kinetics, currently applied in the HEC approach (Kuempel et al., 2001). MPPD-software does not include this specific interstitial compartment (Fröhlich et al., 2016). Kuempel et al. (2015) found that “the estimates from these models differed by a factor of 2-3 with the interstitial-sequestration model predicting lower air borne concentrations associated with the working lifetime retained lung burden”.

Fibrotic effects as observed in humans were not seen with PSLT substances in the rat at low concentrations or only to a marginal degree, whereas fibrotic effects in rats increased significantly only in doses well above the “overload threshold”. From this it could be either concluded i) that the rat is not a suitable model to extrapolate interstitial effects to humans (this question is also raised by Bos et al. (2019)), or ii) that high “overdose” exposure in rats is needed to extrapolate such effects from rodents to humans, or iii) that an interspecies elimination ratio ELR_R/ELR_T well below 0.15 is needed to quantitatively include effects in the interstitium from rat to humans.

Nikula et al. (2001) showed different distributions within lung compartments for two PSLT substances (diesel soot (rats) and coal dust (humans)). The two PSLT substances were compared, as there were no interspecies comparative data for just one single compound available. For the parenchymal lumens in rats no influence on the percentage of retained PSLT depending on exposure concentration was observed (about 80% retention independently from particle concentration), whereas a significant decline of the retained particulate material (from about 40% to about 5%) was found for the human parenchymal lumens of the lung. The profiles for interstitium retentions were quite different: in rats, a low and nearly constant fraction of about 20% was retained by the experimental animals. In humans, the retained volume percentage increased from control to higher concentrations of the particulate material (from about 55% to about 90%). This comparison underlines the different relevance of the interstitium for retention of particles in the two species.

Bevan et al. (2018) analysed particle translocations of PSLT particles in rats and humans. They postulated highly different retention patterns of particles in the two species. The authors claim that this different distribution of the retained particles leads to different toxicity potencies of PSLT particles in the two species. It should be noted,

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10 see figures 5 and 6 in Nikula et al. (2001)

11 Bevan et al. (2018) mainly focus on the relevance of lung cancer as observed in the rat for human risk assessment. This report on HEC methodology does not discuss specifically certain endpoints like cancer and no potential species differences of such effects.
however, that the illustration by Bevan et al. does not include concentration dependent changes in translocation and retention patterns.

Differently from PSLT particles, soluble metal particles like cobalt sulfate caused fibrotic effects in rats already at low concentrations (NTP, 1998). However, for cobalt it is not clear, whether fibrosis is a relevant endpoint of respiratory effects in humans.

Byrne and Baugh (2008) and Dixon (2008) also describe specific evidence of translocation and fibrotic effects in experimental animals and humans in case of nanoparticles. However, also re-appearance of nanoparticles on lung epithelium from the interstitium has been observed and was probably mediated by macrophage-translocation (Geiser and Kreyling, 2010; Riediker et al., 2019). Quantitative estimates on the relevance of this redistribution are not available.

Table 6-1: Schematic of rat and primate/human particle overload, as postulated by Bevan et al. (2018)

<table>
<thead>
<tr>
<th></th>
<th>Burden in rats</th>
<th>Burden in humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free particles in the alveolar space</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Particle-loaded macrophages</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Epithelial hyperplasia</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Interstitial transfer of particles</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

6.5 Clearance impairments at high exposure concentrations

Clearance impairment is usually referring to impairment of mechanical clearance via mucociliary elimination. This impairment becomes obvious by a slower AM elimination rate. The slower AM elimination rate is not necessarily due to AM damage. In recent studies it is postulated that alveolar macrophages are not directly damaged or impaired in their phagocytic activity or increased in volume or less mobile at elevated lung particle loads (Li and Pauluhn, 2018). These authors find that already a low lung particle load leads to increased influx of alveolar macrophages, polymorphonuclear neutrophils (PMNs), and cytokines in the lung (summarized by an increase in “total cell...
count”, TCC), which, in turn, leads to reductions in mucociliary clearance just because of the increased number of cells to be eliminated per unit of time. At high TCC levels, where adaptive responses are not sufficiently protective anymore, there will be inflammatory injury of the lung. Li and Pauluhn (2018) specifically focus to the relevance of PMNs: “neutrophils are primary perpetrators of inflammatory injury in the lung”. An increase of about 4% of PMNs may be indicative for some first adverse effects in the lung. Similarly, an increase of TCC by 6% in the lung may also be indicative for this particle effect, where clearance half-life also increases significantly. The increase in TTC by 6% coincides with the 6% volumetric load of the alveolar macrophages (“displacement volume”). This observation therefore maintains the 6% AM volumetric load from Morrow (1992), but provides only a coincidence by chance with this early figure, which is not mechanistically linked to an impairment of the alveolar macrophages (Section 5.3). This new interpretation of the “overload effect” gives rise to some doubts, i) about the mechanistic interpretation of the “overload” effect, ii) about the justification of the particle volume being the most appropriate dose metric, iii) about the AM volume as being the best unit for normalisation, as it is not clearly mechanistically linked to the observed effects and iv) about impaired mucociliary clearance being the earliest and most significant adverse effect from particle exposure (Section 5.3), while inflammation of lung epithelium may occur at similar concentrations and is more clearly regarded as being an adverse effect. However, other authors have postulated damage of alveolar macrophages from particles and assume that clearance hindrance is not primarily a secondary effect from the increase in TCC (Bos et al., 2019).

It should be noted that an increase in elimination half-life in the rat is not always an indication of a mere particle effect. Particles with some chemical reactivity (from solubility or just particle surface reactivity with biological matrices) can lead to direct damage of the alveolar macrophages and, thus, to impaired clearance. Therefore, it may be premature to assign all clearance impairments at elevated exposure levels to a PSLT effect.

### 6.6 Elimination of soluble particles

In MPPD 2.11 consideration of clearance focussed on poorly soluble particles and could not be changed by the user (Miller et al., 2016). Therefore, MPPD use was limited to PSLT substances or to model deposition. However, in principle, version 3.04 of MPPD includes a formula to calculate clearance, which allows the user to specify values for certain constants as well as for the mucous velocities for the TB region, thereby extending the clearance modelling of MPPD to any type of particle (Miller et al., 2016). However, such “specific constants” to calculate clearance are usually not available, not specified for the two species, and probably would differ for (a) water soluble particles, (b) particles that are soluble in the alveolar lining fluid, (c) particles that are soluble in the lysosomal fluids, (d) particles that are soluble in the interstitium in combination with subsequent reactions with biological matrices, which may further modify clearance velocity. Therefore, it is concluded that MPPD is still not a suitable tool to calculate clearance for the different types of particles with varying bioaccessibility in different regions of the respiratory tract.

As indicated in Section 6.3, the consequences of solubility on clearance time and on the most relevant mode of action are not well known, specifically at intermediate
solubility in physiological fluids. Water solubility is a poor indicator of solubility in the various compartments of the respiratory tract. It is generally assumed that there are no major species differences in elimination half-life from the lung for highly soluble particles, if dissolution determines clearance time (Oller and Oberdörster, 2016). However, even for highly soluble particles, dissolution is not the only determinant of retention and elimination rate, with variable consequences for interspecies differences:

- Cadmium oxide, which is poorly soluble in water, is eliminated in animal species at a similar rate as is the highly soluble cadmium chloride (Oberdörster, 1988).
- The highly soluble cadmium chloride is eliminated much faster in rats compared to dogs or monkeys (Oberdörster, 1988).
- Tricobalt tetraoxide, which is moderately soluble in water (1.6 mg/L in water, 20°C) is eliminated much faster in rats, hamsters and mice compared to dogs, guinea pigs, baboons, and men (Bailey et al., 1989).
- Particle size may greatly influence solubility: as shown for tricobalt tetraoxide in vitro, intracellular solubility resulting in substantially different dissolution rates after 2 weeks, when 50% vs. 5% vs. 3% vs. 2% of the original particle mass for 0.3 or 0.7 or 0.8 or 1.7 µm-particles, respectively, were solubilised (Kreyling et al., 1990)
- Even highly soluble particles may be translocated and retained in the interstitium: the highly soluble cobalt sulphate is predominantly eliminated to the interstitium in rats resulting in long-term effects in this tissue, but similar elimination kinetics in humans are only observed for insoluble cobalt compounds or tungsten carbide alloys (NTP, 1998; 2014), with insufficient human data for cobalt sulphate
- Chemically active soluble substances may damage alveolar epithelial cells, which leads to major changes in elimination kinetics. Theoretically, the alveolar epithelium could be damaged to a degree that solute clearance becomes limited by the endothelial barrier of the pulmonary capillaries (Oberdörster, 1988)
- In impaired lung tissue (e.g. from smokers) elimination of soluble particles is increased due to effects on the alveolar epithelial barrier of mediators released from activated AM and solid particle clearance decreased, due to impaired AM function (Oberdörster, 1988)
- Adsorption of soluble particles to other particles (e.g. benzo(a)pyrene to diesel particles) or to endogenous biomolecules (like metallothionein, enzymes like phosphatase) may significantly alter elimination rate time of particles and MoA of pulmonary effects (Galle et al., 1992; Oberdörster, 1988), e.g., for metal compounds (Beyersmann and Hartwig, 2008).
- Nanoparticles with different solubility properties were analysed for their elimination kinetics. For example, slow dissolution (abiotic dissolution ≪30% per 7 days, or even no apparent dissolution) of barium sulphate and silica dioxide was followed by re-precipitation and transformation. In contrast, e.g., zinc oxide or copper oxide showed high dissolution and clearance (abiotic dissolution ranging from 30% to 100% after 7 days) Particle size had a relevant influence on dissolution properties (Koltermann-Jüllly et al., 2018).

As mucociliary clearance by alveolar macrophages contributes to elimination of moderately soluble particles and as also readily soluble particles may be eliminated in
parts via AM transport, if those reacted with endogenous proteins, there is probably only a small portion of particles where no species differences are expected. However, there are only few adequate lung clearance data available for interspecies comparisons on clearance and conclusions are, therefore, highly uncertain.

6.7 Variation in clearance due to respiratory illnesses, individual differences and local inhomogeneity

Average elimination data and average interspecies elimination rate ratios (as provided by ELR\textsuperscript{H}/ELR\textsuperscript{T}) may not be representative and meaningful, if, in truth, local elimination at certain hot spots determines respiratory effect potency (Sections 4.5 and 5.1). For hot spots and PSLT-particle elimination Donaldson et al. (2008) suggests the alveolar region proximal to those hot spots (PAR) to be more adequate to calculate meaningful species differences: “The proximal alveolar region (PAR) of the lung has been identified as a key site for the retention of respirable particles, as it receives high deposition but has slow clearance compared to the larger airways” (Donaldson et al., 2008).

In addition, it should be noted that there is individual variability in elimination due to personal airway anatomy, breathing patterns and potential airway impairments from respiratory illnesses. “ICRP recommends reducing the clearance rate by a factor of two when estimating the retained particle dose among individuals with COPD” (Kuempel et al., 2015). There exists no adequate aggregate information about the variability in clearance rate in experimental animals and the consequences of this variability on ELR\textsuperscript{H}/ELR\textsuperscript{T} – ratio variability. Within the framework of this study, no substance specific data on clearance variability in rats were retrieved and statistically analysed.

6.8 Interspecies differences in elimination rate from mice experimental studies

Elimination half-life data from the lung of mice for PSLT particles are reported in literature (Benson et al., 1995; Snipes, 1989; Snipes et al., 1989). However, there are only limited data available, which may be less representative than those for the rat. Examples for very similar elimination rates for PSLT particles as compared to rats are provided in literature (Snipes, 1989). Changes of half-life due to solubility have to be assessed case-by-case. There is more evidence for mice than for rats that particles are translocated to the interstitium.

For the purpose to calculate an example HEC in this report (mice to human – extrapolation), we assume an identical elimination half-life for mice and rats (Section 7.8.4).
6.9 Summary and conclusions on retention and elimination

The current German HEC procedure (AGS, 2013) to calculate ELRH/ELRT for PSLT substances with a default rate of 0.15 is apparently quite conservative, if elimination to the interstitium is not considered. In this case, the factor could be increased to 0.2 (=60/300), due to shorter clearance time in humans. However, because of significant species differences in translocation to the interstitium and potential interspecies differences in adverse effect potency in the interstitium, these values for ELR are highly uncertain, even for PSLT particles and also at lower concentrations (below concentrations that lead to impaired AM clearance). Therefore, in addition to a default ELRH/ELRT of 0.2 for AM clearance, the probability of relevant effects of PSLT particles in the interstitium should be considered in both species, which may increase interspecies differences.

For particles with low solubility, which are, however, chemically reactive in the alveolar region, there are insufficient data to conclude on a default factor. If clearance time in the rodent is increased (i.e. significantly higher than 60-90 days), this may be due to general high dose particle effects associated with impaired AM clearance and due to increased TCC in the alveolar region (“overload effects”), but it may also be due to chemically induced damage of macrophages or epithelial cells with subsequent reduced elimination. It is often not known whether identical reductions in elimination rate takes place in rats and in humans.

Similarly, for substances with intermediate solubility in physiological fluids or intermediate water solubility the reduction in species differences is not well known, but it is obvious that the mode of action may be different compared to PSLT particles. Probably, more than one MoA will be relevant. There are no sound quantitative data to calculate a default ELRH/ELRT for substances with intermediate solubility. Therefore, if a default has to be selected, this could only be quantified pragmatically, because of the significant quantitative uncertainties. This does not preclude that adequate interspecies comparisons in elimination are available in individual cases. However, such case-by-case discussions always should consider the uncertainties from translocation of particles to the interstitium in either species.

Finally, for highly soluble particles

i) the quantitative solubility in either epithelial lining fluid, lysosomal fluid, or interstitial fluid has not yet been determined adequately to conclude definitely on equal elimination rates (i.e. ELRH/ELRT = 1). Therefore, any chosen solubility value would be rather arbitrary and associated with relevant uncertainty,

ii) again, also soluble particles may be translocated to the interstitium and potential species differences need to be considered for this compartment,

iii) there may be many reasons to deviate from a default with no differences in clearance time, if there are indications of a binding to proteins or other alveolar tissue, which usually are associated with species differences.
It should be emphasised that there may be other clearance mechanisms but just AM mechanical clearance and that species differences are not limited just to AM clearance.

Considering all these bits of information on elimination, it could also be justified to abstain to select a separate $\text{ELR}_h/\text{ELR}_T$ – ratio. Instead an overall uncertainty factor, which addresses normalisation and elimination simultaneously, without pretending exact scientific background, could be thought of. Such an “aggregate 3 ratios” approach is outlined in Section 7.4.
7 Aggregated HEC-Calculation

7.1 Aggregated HEC-calculation – overview

In the previous Sections we discussed the single four interspecies ratios (weighted breathing volume, normalisation factor, elimination rate, and deposition fraction), which determine HEC. Finally, those ratios are multiplied for aggregation. However, some characteristics are to be acknowledged for this HEC-result:

- Differently from “allometric scaling” for systemic effects, the HEC calculation is significantly influenced by the assumed mode of action. This aspect is briefly discussed in Section 7.2.
- Not always all the four ratios mentioned above can be determined from the experimental data. Therefore, it is sometimes suggested “to take what we have” and neglect those ratios, for which no data are available, i.e., calculate a “partial HEC”. We discuss this proposal in Section 7.3.
- Normalisation factor and elimination rate are interrelated terms, as both are influenced by particle solubility and mode of action. Therefore, an alternative approach is discussed: instead of the traditional “4 ratios approach” for HEC, the human equivalent concentration may also be estimated by an “aggregate 3 ratios approach”, combining the normalisation factor and elimination rate. This alternative is suggested in Section 7.4.
- MPPD 3.04 permits to calculate HEC based on experimental animal inhalation studies with mice. In Section 7.4 we bring together the various data for mice as a starting point to calculate HEC and discuss the consequences.
- As indicated in Section 6.4, elimination rate is different, if translocation to the interstitium is included or excluded from assessment considerations. It is suggested to add an additional assessment factor to address potential translocation to the interstitium. For discussion see Section 7.6.
- The applicability of HEC calculation results may be restricted because of overall uncertainties. Such an applicability constraint is suggested in Section 3.
- Some HEC examples are calculated both, with the “4 ratios approach” and with the “aggregate 3 ratios approach”, to analyse the quantitative consequences of the updates in Sections 3 to 6, and specifically about the application of MPPD 3.04 (Section 7.8).
- Finally, main uncertainties of the HEC approach are summarized in Section 7.9.

7.2 HEC – no isolated precursor step

From the determinants described above (Sections 3 to 6) it is obvious that HEC calculation for respiratory effects in the lower respiratory tract is not a routine procedure, which could be executed adequately without a very good understanding of the mode of action for the observed effects. For HEC calculation qualified information
is needed for all four aspects (weighted breathing volume, deposition fraction, normalising factor and retention) for both species. Data compilation and analysis may be more complex than application of allometric caloric demand scaling, which is one step within interspecies extrapolation for systemic effects.

7.3 Partial HEC, if only selected data are available?

As described above (Section 7.2), HEC is a composed term. Frequently, we are not able to quantify all of the ratios \((\frac{A_{gV_T}}{A_{gV_H}}, \frac{N_{F_H}}{N_{F_T}}, \frac{E_{LR_H}}{E_{LR_T}}\) and \((\frac{D_{F_T}}{D_{F_H}}))\) with similar precision or, in cases of high uncertainty, it may even be impossible to quantify some of the ratios at all. Usually there should be sufficiently qualified information on weighted breathing volume ratios and on the deposition fraction ratio, but information for normalisation and retention (in combination with solubility and mode of action) may be inadequate.

There are three different options, if such significant uncertainties prevail:

- **Option 1:** Set HEC/cT = 1
- **Option 2:** Use the deposited dose normalised to the alveolar surface and assume no species differences in elimination rates
- **Option 3:** Combine the ratios, which are uncertain in quantification and apply an aggregate pragmatic assessment factor to cover limited information on normalisation, solubility and retention species differences.

**Option 1:**
Using HEC/cT = 1 means that exposure to air concentrations [mg/m³] is assumed to be equipotent for the experimental animals and humans without interspecies corrections. It is suggested to apply this option (HEC/cT=1), if there is no convincing evidence that one of the ratios with insufficient data needs to be accounted for explicitly. Moreover, after calculating HEC for a set of example substances below (Section 7.8), we found that HEC/cT = 1 can frequently be selected, if the overall pursued protection goal is moderate. Balancing the various uncertainties in quantification of the single ratios of the HEC formula on the one side and the limited deviations of the complete HEC result from HEC/cT = 1 on the other side, the assumption that exposure to air concentrations [mg/m³] is equipotent for the experimental animals and humans without corrections for species specific breathing volume, deposition, normalisation and retention may be a reasonable conclusion. If, however, an elevated protection goal is pursued, an additional assessment factor could be considered.

**Option 2:**
Some inhalation toxicologists suggest to assume equal elimination rates \((E_{LR_H}/E_{LR_T} = 1)\) for particles with some solubility and assume normalisation only to the alveolar
surface. This means to use HEC for the deposited dose, instead of the HEC for the retained lung burden. However, this approach is not supported, unless there is substantial evidence that there are no species differences in elimination half-life (which includes not only AM clearance, but also other clearance mechanisms – see Section 6.2) or unless the MoA is clearly linked to the deposited dose instead of the retained dose. We advise against a default selection of ELR_{H}/ELR_T =1, because in this case the overall probability to create a substantial bias from the “real” HEC is regarded as higher compared to select option 1 (i.e. HEC/c=1).

**Option 3:**
There is a third option to estimate HEC, if the quality of the data is insufficient to quantify some of the sub-factors and if option 1 (i.e. HEC/c_T=1) is regarded not sufficiently protective. This third approach substitutes the single ratios for normalisation and elimination species differences (NF_{H}/NF_T, ELR_{H}/ELR_T) by a pragmatic aggregate assessment factor based on the retained dose (“aggregate 3 ratios approach”; Section 7.4).

**7.4 Suggested “aggregate 3 ratios approach”**

For weighted breathing volumes (Section 3) and deposition fractions (Section 4), default HEC calculations for extrapolation from rat to man can be readily performed with limited additional guidance to be developed. The use of MPPD (version 3.04) is suggested for calculation of deposition fractions.

However, the application of HEC for the ratios (NF_{H}/NF_T and ELR_{H}/ELR_T) for interspecies assessments based on rat data (Sections 5 and 6) should be reconsidered, because of significant overall uncertainties. Acknowledging those uncertainties and the complex interrelation of MoA and particle solubility on the one side and the corresponding normalisation and interspecies elimination rate ratio quantification on the other side, we suggest a pragmatic aggregate approach for those ratios in the HEC formula in combination, as outlined below. The problem arising from translocation of particles to the interstitium, which may also affect normalisation and elimination rate is discussed separately (Section 7.6).

Firstly, we recall the quantitative uncertainties on normalisation and elimination rate quantification and the parameters influencing those uncertainties. We combine this information with some suggestions to be reflected in the aggregate approach:

- **Alveolar surface area** is a frequently suggested unit for normalisation. Quantitative figures for NF_{H}/NF_T ratio are in the range of 140 – 350 (Table 5-1). The difference is partly due to the inclusion or exclusion of the TB region, with no precise generic answer possible, whether the lower TB region should be included or not. Lung surface is regarded as adequate unit for normalisation by many assessors. Only for PSLT particles there are some strong indications that lung surface should not be justified for normalisation. This holds also true for soluble particles. Specifically, for substances readily soluble in alveolar lining fluids the reference to lung surface should be considered. Acknowledging the various uncertainties an average normalisation factor ratio of 250 can be
considered based on lung surface. This value is also close to the lung surface estimate by Kuempel et al. (2015).

- Total alveolar macrophage volume is frequently suggested for normalisation with respect to impaired clearance effects by PSLT particles. The range of suggested volume data NFH/NFT ratios is in the range of 280-1110 (Section 5.4; Table 5-2). It should be acknowledged that macrophage clearance and the species differences in particle clearance may also be relevant for non-PSLT particles and soluble particles. Specifically, for substances readily soluble in lysosomal fluids the reference to AM volume should be considered. Acknowledging the various uncertainties an average normalisation factor ratio of 750 can be considered based on total AM volume. This value is also close to the AM volume estimate by Kuempel et al. (2001).

- Therefore, if normalisation is *not clearly only* to the total alveolar macrophage volume, but also the lung surface is to be considered, some factor within the range from 250 to 750 should be used to account for different optimal normalisations depending on the various mode of actions. From this we assign pragmatically
  - a normalisation factor ratio of **600**, if a **major influence** of the alveolar macrophages on optimal normalisation is supported, and a
  - normalisation factor ratio of **400**, if a **major influence** of the lung surface on the optimal normalisation is supported.

- The current default in Germany for elimination rate is based on clearance half-life differences for PSLT substances in the rat (40-90d; usually set to 60 days) and in humans (400 d as current default for PSLT). This results in an interspecies default elimination rate ratio of 0.15 (=60/400). However, as has been recently demonstrated, the AM clearance of particles in humans is usually faster (about 300 d). In addition, additional translocation to the interstitium may have to be considered, which would increase the overall elimination half-life in humans well above the former 400 days. As we exclude interstitium translocation in this aggregate 3 ratios HEC approach (focusing on normalisation and elimination) and assign a separate step to this issue (Section 7.6), we can exclude this prolongation in half-life. We, therefore, suggest a slightly increased default interspecies elimination rate ratio of 0.2 (=60/300; Sections 6.4 and 6.9). There are no qualified data on elimination rate species differences based on other MoA apart from PSLT AM clearance impairment. Specifically, for soluble particles, which are not definitely readily eliminated, some species differences are to be expected, but are not yet sufficiently assessed to provide a sound default elimination rate for interspecies comparisons. For “some” solubility impact on elimination rate is considered ELR_H/ELR_T ratio will be in the range of >0.2 and <1.

From this we apply pragmatically
  - ELR_H/ELR_T of 0.25 for substances with low solubility
  - ELR_H/ELR_T of 0.5 for substances with intermediate solubility
  - ELR_H/ELR_T of 0.8 for substances with high solubility,
to account for species differences in elimination rate, if solubility has “some” impact on elimination duration. We further assume that \( \text{ELR}_{\text{P}}/\text{ELR}_{\text{T}} \) is never equal to the upper or lower limit of the range, i.e. never \( =0.2 \) or \( =1 \). This means, that minor species differences are assumed also for substances with high solubility and some solubility is also assumed for substances with low solubility.

Aggregate approach (aggregate “normalisation and elimination rate ratio”, NEIR):

We suggest to establish an aggregate term to consider interspecies differences in normalization and elimination and call this ratio \textbf{NEIR}. The following situations may be discriminated:

- **Normalisation predominantly but not exclusively** to alveolar macrophages volume (i.e., NF-ratio: 600), and intermediate solubility (i.e, ELR–ratio: 0.5): \( \rightarrow \) NEIR will be set to \( 600 \times 0.5 = 300 \)

- **Normalisation predominantly but not exclusively** to lung epithelial surface (i.e., NF-ratio: 400), and intermediate solubility (i.e., ELR-ratio: 0.5): \( \rightarrow \) NEIR will be set to \( 400 \times 0.5 = 200 \)

- **Normalisation exclusively** to lung epithelial surface (i.e., NF-ratio: 250), and high solubility (i.e, ELR – ratio: 0.8): \( \rightarrow \) NEIR will be set to \( 250 \times 0.8 = 200 \)

- **Normalisation exclusively** to alveolar macrophages volume (i.e., NF-ratio: 750), and low solubility (i.e., ELR-ratio: 0.25): \( \rightarrow \) NEIR will be set to \( 750 \times 0.25 \approx 200 \)

To demonstrate that the last calculation is sufficiently protective, the value for NEIR could also be interpreted as \( 1110 \times 0.2 \approx 200 \) for NEIR (PSLT particles), with slightly different selected parameters for AM volume (NF ratio from Table 5-1) or elimination rate (ELR ratio from Section 6.9).

Based on the considerations above, the following Table 7-1 shows case-specific NEIRs, which can be either 200 or 300. However, quantitative figures to classify solubility are to be assigned yet.
Table 7-1: Suggested normalisation and elimination interspecies ratio (NEIR) for normalisation and elimination within HEC calculation (aggregate 3 ratios approach)

<table>
<thead>
<tr>
<th>NEIR assignment</th>
<th>Lysosomal Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤# mg/l</td>
</tr>
<tr>
<td>Solubility in Water</td>
<td>Solubility in ALF</td>
</tr>
<tr>
<td>&lt;# mg/l</td>
<td>&lt;# mg/l</td>
</tr>
<tr>
<td>&gt;# g/l</td>
<td>300</td>
</tr>
<tr>
<td>&gt;# g/l</td>
<td>irrelevant</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Green: dominated by AM-volume normalisation and relevant species differences in clearance
Red: mixed, lung-surface area plus AM-volume normalisation and some species differences in clearance
Yellow: lung surface normalisation (because of high solubility) and no relevant indications of species differences

7.5 Aggregated HEC calculation based on experimental data for mice

From the data reported in Sections 3.4, 4.11, 5.6, and 6.8 it is concluded that no default HEC procedure should be proposed, if experimental data from mouse studies are considered as a starting point for interspecies extrapolations for particles. Usually, experimental data from rat should be preferred, if adverse health effects in the lower respiratory tract from particles are to be assessed. The background of this suggestion are overall uncertainties:

- Considerable variability exists in breathing volume data with current default values on tidal volume and breathing frequency mainly just based on two strains (BALB/c- and B6C3F1-mice).
- No direct functional residual capacity data (FRC) for mice were available, but were indirectly derived from rat data (Asgharian et al., 2014; Hsieh et al., 1999).
- There is a dramatic difference, if either normalised to the lung epithelial surface or to the alveolar macrophage volume, with no unambiguous decision criteria, which of the normalisation references should be applied (Section 5.6).
- Documented alveolar surface area data for mice are highly divergent (Section 5.6).
- Few data on representative elimination half-life, specifically, if particle clearance is not only by AM-mucociliary clearance.
- No specific data are available for inhomogeneity in deposition and retention for mice (“hot spots”).
The exclusion or inclusion of the TB-region in addition to the PU-region may be even more relevant for mice compared to rats, because of the generally high particle fraction, deposited in the TB-region in this species. Uncertainties are also shown in quantitative example calculations (Section 7.8.4).

### 7.6 Translocation to the interstitium: suggested separate sub-factor in HEC default calculations

As indicated above (Section 6.4), species differences due to translocation of particles to the lung interstitium are not covered in most former HEC approaches and in the suggested default calculation procedure in this report, so far. However, a separate factor to address this issue is suggested in this Section. From the work by Kuempel et al. (2015; 2001) and by Gregoratto et al. (2010; 2011) it is concluded that interspecies differences from clearance of particle to the lung interstitium should be included in HEC calculations, if relevant. However, there are major uncertainties, i) whether interstitial effects in humans are sufficiently represented in animal studies, i.e. whether the rat is a suitable model animal to provide information on interstitial effects in humans, ii) whether the interstitial effects observed in rats at relatively low exposures only with soluble metal particles are representing relevant effects in human exposure and iii) whether interstitial effects at “overload doses” in rats of PSLT substances are quantitatively comparable to interstitial effects in humans (Bos et al., 2019). However, there is clear evidence that elimination half-life of particles in humans is significantly increased, if the interstitium is included as part of the pulmonary region (multi-phase elimination). Further discussion on interstitial effects from particles, translocation and species differences are provided in Section 6.4. In order to cover respective differences with respect to deposition, normalisation, and clearance, we suggest to apply an additional interspecies sub-factor of 0.5 (IIF = interstitium interspecies factor) within the HEC-procedure, if there is any specific indication, that interstitium effects may be relevant in either species for a certain particle assessment.

“Any specific indication” means, that either in humans (qualitative or quantitative) fibrotic effects are associated with exposure to this particle or if fibrotic effects or (malignant or benign) tumours in interstitial tissue are observed in laboratory animals above control background rate. It is not necessary that such interstitial effects are shown in both species in interspecies comparisons, to justify the need of an IIF.

The suggested factor of 0.5 is taken from an analysis by Kuempel et al. (2015). The authors compared different models and retained lung doses, which differed, whether the interstitium was explicitly addressed or excluded, and found “the estimates from these models differed by a factor of 2-3 with the interstitial-sequestration model predicting lower airborne concentrations associated with the working lifetime retained
Because the inverse of this factor (2-3) is applied within the HEC-formula, IIF is set to $\frac{1}{2} = 0.5$.

Therefore, the default HEC-formula within the **4 ratios approach** or the **aggregate 3 ratios approach** would be modified according the suggestions from Section 7.4

$$HEC/c_T = \frac{(AgV_T / AgV_H) \times (NF_H / NF_T) \times (ELR_H / ELR_T) \times (DF_T / DF_H) \times IIF}{},$$

$$HEC/c_T = (AgV_T / AgV_H) \times NEIR \times IIF \times (DF_T / DF_H)$$

with IIF only, if applicable. Note that the **4 ratios approach** therefore changes to **5 multipliers** and the **aggregate 3 ratios approach** changes to **4 multipliers**, without changes in the terminology.

### 7.7 Range constraints for the HEC-approach

Most of the aspects to be covered by the HEC-calculation have been addressed above (Sections 7.2 to 7.6). However, some major uncertainties remain:

- the consequences of inhomogeneous distribution (hot spots),
- the possible hygroscopic particle growth effects,
- the potential difference in particle size distribution in human vs. experimental animal exposure.

There are no qualified approaches available, how to address the mentioned additional uncertainties quantitatively. We therefore suggest to limit any default HEC – applicability to an upper $HEC/c= 1$, because of overall considerations on protective assessment factors.

We therefore suggest to calculate HEC within the **4 ratios approach**:

a) within the **4 ratios approach**:

$$HEC/c_T = \frac{(AgV_T / AgV_H) \times (NF_H / NF_T) \times (ELR_H / ELR_T) \times (DF_T / DF_H) \times IIF}{},$$

if $HEC/c_T < 1$ and

$$HEC/c_T = 1,$$

if $(AgV_T / AgV_H) \times (NF_H / NF_T) \times (ELR_H / ELR_T) \times (DF_T / DF_H) \times IIF$, if $HEC/c_T \geq 1$

b) or within the **aggregate 3 ratios approach**:

$$HEC/c_T = (AgV_T / AgV_H) \times NEIR \times IIF \times (DF_T / DF_H),$$

if $HEC/c_T < 1$ and

$$HEC/c_T = 1, \text{ if } (AgV_T / AgV_H) \times NEIR \times IIF \times (DF_T / DF_H), \text{ if } HEC/c_T \geq 1$$

Note that this is a suggestion for default HEC calculations. It is always accepted to deviate from default in case of qualified data and case-by-case justification.
7.8 Some examples for HEC

7.8.1 HEC - calculation from rat data for PSLT substances: titanium dioxide

This example is selected in order to compare the results from HEC calculation by Pauluhn (2011a) and by Hartwig (2012) with the respective updated considerations, as documented and discussed in the present report. For titanium dioxide only the study by Muhle et al. (1991) was used for parameter specification. It is not intended to present an overall assessment on titanium dioxide, but only to compare the HEC-transformed NOAEC in rats with the corresponding human NAEC.

Input parameters:

NOAEC (rat) = 5 mg/m³
MMAD: 1.1 µm
GSD: 1.6
Density: 4.3
Exposure 6h/d; 5d/w; 2 years
Strain: F344 rats
Body weight: no data provided; used: 370 g (F344 male, 12 months, (Mauderly, 1986))
MPPD calculation of deposition: version 3.04

Results:

1.) AgVT/AgVH

Non-default:
Breathing scenario rat in the respective original study: whole body exposure (not: nose only)
Breathing frequency: 109.0787 bpm according to MPPD exposure for given body weight
Tidal volume: 2.59 mL according to MPPD exposure for given body weight

تذكر: 101.706 l/d Breathing volume = 0.102 m³/d
101.706 l/d x 5/7 = 0.073 (chronic weighted breathing volume, rat)
0.073 (this calculation, rat)/ 6.57 (average human) = 0.011 = AgVT/AgVH

The calculated AgVT/AgVH differs only slightly (factor ≈ 1.4) from the former value (0.008).
2.) DF\textsubscript{T}/DF\textsubscript{H}

DF\textsubscript{T}/DF\textsubscript{H} calculated as shown in Table 7-2. We limit subsequent calculations to the pulmonary region, which results in a DF\textsubscript{T}/DF\textsubscript{H}-ratio of 0.692. This ratio is close to identical to the parallel calculation with MPPD 2.11 (MPPD 2.11; PU-DF\textsubscript{T}/DF\textsubscript{H} = 0.695; data not shown). Note, however, that the two calculations would lead to some difference, if the TB-region would be included, i.e. MPPD 3.04: PU+TB-DF\textsubscript{T}/DF\textsubscript{H} = 0.89 vs. MPPD 2.11: PU+TB-DF\textsubscript{T}/DF\textsubscript{H} = 0.68). There probably is some influence in the deposition calculation with “whole body exposure” vs. “nose only” exposure; this distinction is only possible in MPPD 3.04 and is based on a different allometric calculation compared to the “nose only” scenario. MPPD 2.11 does not permit to differentiate “nose only” vs. “whole body” – exposure.

Table 7-2: Example calculation of deposition fractions and DF\textsubscript{T}/DF\textsubscript{H} for Titanium dioxide (data from Muhle et al., (1991)), MPPD 3.04

<table>
<thead>
<tr>
<th>MPPD 3.04</th>
</tr>
</thead>
<tbody>
<tr>
<td>PU</td>
</tr>
<tr>
<td>DF\textsubscript{H}</td>
</tr>
<tr>
<td>DF\textsubscript{T}</td>
</tr>
<tr>
<td>DF\textsubscript{T}/DF\textsubscript{H}</td>
</tr>
</tbody>
</table>

3.) Normalisation

In most former approaches, PSLT particles were normalised to the alveolar macrophage volume. This normalisation reference is confirmed for PSLT in this report. According to Pauluhn (2011a) (Table 5-2, this report), a normalising factor NF\textsubscript{H}/NF\textsubscript{T} of \(\approx 1110\) would be used. Instead, some other data provided in Table 5-2 within the range of 278 and 1110. For the purpose of this example calculation we use an average value of 700 ((278+1110)/2\(\approx\)700) for the 4 ratios approach in HEC calculation.

4.) Retention

The traditional retention factor ratio is derived from the elimination half-life from the lung in humans (400 d) and the respective value for rats (60 d). However, as discussed in Sections 6.4 and 6.9, human lung elimination half-life is reduced, if the interstitium compartment is excluded as part of the target organ (lung), with ELR\textsubscript{H}/ELR\textsubscript{T} = 0.2 instead of ELR\textsubscript{H}/ELR\textsubscript{T} = 0.15 for PSLT-particles. This is not the case for titanium dioxide, where interstitium effects were observed in the rat (although only at higher exposures). Therefore, according to Section 7.6, an additional factor of 2 (IIF= 0.5) is suggested to be used supplementary in both, the “4 ratios approach” and the “aggregate 3 ratios approach”.
5.) Overall HEC

This example assessment provides a HEC for titanium dioxide, based on the study by Muhle et al. (1991). If each single factor is quantified (4 ratios approach) this results in the following HEC:

$$\text{HEC} = 0.011 \times 0.692 \times 700 \times 0.2 \times 0.5 \times cT = 0.53 \times cT$$

If the aggregate 3 ratios approach (Section 7.4) with a NEIR of 200 is used (as depicted from Table 7-1),

$$\text{HEC} = 0.011 \times 0.692 \times 200 \times 0.5 \times cT = 0.76 \times cT$$

With a NOAEC_{rat} of 5 mg/m³ in experimental animals these two calculations result in similar values of NAEC_{HEC} = 2.66 (4 ratios approach) – 3.81 mg/m³ (3 ratios approach).

6.) Discussion

Calculations for titanium dioxide based on the identical study performed by Hartwig (2012) resulted in a NAEC_{HEC} = 1.06 mg/m³ (rounded to 1.1 mg/m³; Section 5.2). Hartwig used the lung surface for normalisation, which resulted in smaller values. Minor additional differences are due to the additional inclusion of IIF because of the interstitium effects. Detailed MPPD reports were not available and an earlier version of MPPD was used by Hartwig (2012). These values (2.66-3.81 mg/m³) are slightly higher than the threshold calculated with approach B in Section 5.2 (2.15 mg/m³ for a density of 4.3), but in good agreement with the former calculation. It also considers possible interstitium effects, which were not included previously. The aggregate 3 ratios approach provides similar results as the more detailed 4 ratios approach.

7.8.2 HEC – calculation from rat data for water-soluble particles: cobalt sulfate

This example illustrates the use of MPPD version 3.04 in combination with further updates in HEC calculation discussed in the present report for a substance, which is soluble in water (337.4 g/Liter at 20°C), and also soluble in lysosomal fluid and in epithelial lining fluid (AGS, 2017). The experimental data, as reported by NTP (1991; 1998) for cobalt sulfate heptahydrate were used for parameter specification and transformed to cobalt sulfate, if applicable. It is not intended to present an overall assessment on cobalt sulfate, but only to compare the HEC-transformed NOAEC in rats with the corresponding human NAEC.

Input parameters:

- NOAEC (rat) = 67 µg/m³ (no pulmonary adverse effects in a subchronic study) (NTP, 1991)
- MMAD: 1.4 µm (NTP, 1991)
- GSD: 2.1 (NTP, 1991)
- Density: 2.0 g/cm³ (Zalkin et al., 1961)
- Exposure 6h/d; 5d/w
Strain: F344 rats
Body weight: 434 grams (NTP, 1998)
MPPD calculation of deposition: version 3.04

Results:

1.) $\frac{AgV_T}{AgV_H}$

Breathing frequency: 166 (usually for SD-rats, adopted)

Tidal volume: 3.24 according to MPPD, exposure for given body weight

$\Rightarrow 193622 \text{ l/d Breathing volume} = 0.193 \text{ m}^3/\text{d}$

$\Rightarrow 0.193/6.57 = 0.029$

$\Rightarrow 0.029 \times 5/7 = 0.021 = \frac{AgV_T}{AgV_H}$

The calculated $\frac{AgV_T}{AgV_H}$ differs by a factor of 2.6 from the former value (0.008)

2.) $\frac{DFT}{DFH}$

$\frac{DFT}{DFH}$ was calculated as shown in Table 7-3. We limit subsequent calculations to the pulmonary region, which results in a $\frac{DFT}{DFH}$-ratio of 0.33. Uncertainties in deposition come from hygroscopic growth for water-soluble particles, but were not considered in this calculation.

Table 7-3:  Example calculation of deposition fractions and $\frac{DFT}{DFH}$ for cobalt sulfate (data from NTP (1991; 1998)), MPPD 3.04

<table>
<thead>
<tr>
<th></th>
<th>MPPD 3.04</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PU</td>
</tr>
<tr>
<td>$DF_H$</td>
<td>0.0944</td>
</tr>
<tr>
<td>$DF_T$</td>
<td>0.0313</td>
</tr>
<tr>
<td>$\frac{DFT}{DFH}$</td>
<td>0.33</td>
</tr>
</tbody>
</table>

3.) Normalisation

In most former approaches, normalisation ratio for water-soluble particles would have been to the alveolar surface area ($NF_H/NF_T$). Quantitative figures (Table 5-1) are in the range from 140 to 349 for $NF_H/NF_T$. For the purpose of this example, we use an average value of 250 ($\frac{(349+140)}{2} \approx 250$) for the 4 ratios approach HEC- calculation.

4.) Retention

The retention factor in the updated HEC-approach is derived from the elimination half-life from the lung in humans (300d) and the respective value for rats (60d). However,
for water-soluble substances, no species differences are assumed (ELR_H/ELR_T=1). However, as discussed in Sections 6.4 and 6.9, species effects in retention are expected, if the interstitium compartment is included. This is the case for cobalt sulfate, where interstitium effects were observed in the rat (NTP, 1998). Therefore, according to Section 7.6, an additional factor of 2 (IIF= 0.5) is suggested for both, the **4 ratios approach** and the **aggregate 3 ratios approach**.

However, although water-solubility of cobalt sulfate is high, there is evidence that the substance binds to proteins: “... *in vivo*, the bioavailability of free Co(II) is expected to be relatively limited, because these cations precipitate in the presence of physiological concentrations of phosphates” (Paustenbach et al., 2013) and also supported by further *in vitro* – observations (Stopford et al., 2003). This is not considered in the **4 ratios approach**. If the **aggregate 3 ratios approach** is used, NEIR of 300 is suggested for such substances according to the scheme in Section 7.4.

5.) **Overall HEC**

This example assessment provides a HEC for cobalt sulfate, based on the study by NTP (1991; 1998). If each single factor is quantified (**4 ratios approach**) this results in the following HEC:

\[
\text{HEC} = 0.021 \times 0.33 \times 250 \times 1 \times 0.5 \times c_T = 0.86 \times c_T
\]

If the **aggregate 3 ratios approach** of Section 7.4 with NEIR = 300 is used,

\[
\text{HEC} = 0.021 \times 0.33 \times 300 \times 0.5 \times c_T = 1.04 \times c_T, \text{ changed to } \frac{\text{HEC}}{c_T} = 1 \text{ (according to Section 7.7)}
\]

According to Section 7.7, \( \frac{\text{HEC}}{c_T} > 1 \) are not permitted in the suggested update, because of overall uncertainties. Therefore, in the aggregate approach, \( \frac{\text{HEC}}{c_T} \) is set to 1 (and NOAEC_{rat} = NAEC_{HEC}).

In consequence, with the 4 ratios approach

\[
\text{HEC} = 0.86 \times 67 \ \mu\text{g/m}^3 = 58 \ \mu\text{g/m}^3,
\]

With the 3 ratios approach, the NOAEC rat is maintained as the NOAEC for humans:

\[
\text{HEC} = 67 \ \mu\text{g/m}^3.
\]

6.) **Discussion**

Note that the **4 ratios approach** and the **aggregate 3 ratios approach** come up with very similar results close to HEC/cT = 1.

7.8.3 **HEC – calculation from rat data for particles with lysosomal solubility: cobalt metal**

This example illustrates the use of MPPD version 3.04 in combination with further updates in HEC calculation discussed in the present report for a substance, which is poorly soluble in water (2.9 mg/Liter at 20°C) and in epithelial lining fluid (4.8 % solubility for extra fine particles at pH 7.4), but soluble in (macrophage) lysosomal fluid (92.4 % solubility for extra fine particles, at pH 4.5) (AGS, 2017). This example is
performed with parameters for rats and can be compared to the example below (Section 7.8.4) with mice. The experimental data, as reported by NTP (2014) for cobalt metal were used for parameter specification. It is not intended to present an overall assessment on cobalt metal, but only to compare the HEC-transformed NOAEC in rats with the corresponding human NAEC.

**Input parameters:**
LOAEC (rat) = 1.25 mg/m³
MMAD: 1.8 µm
GSD: 1.7
Density: 8.81 g/cm³
Exposure 6h/d; 5d/w
Strain: F344 rats
Body weight: 434 grams (assumed identical to the cobalt sulfate study, within the range of body weights, male rats, for cobalt metal)
MPPD –calculation of deposition: version 3.04

**Results:**

1.) \( \frac{AgVT}{AgV_H} \)
Breathing frequency: 166 (usually for SD-rats, adopted)
Tidal volume: 3.24 mL according to MPPD, exposure for given body weight

\[
\begin{align*}
\Rightarrow & \quad 193622 \text{ mL/d Breathing volume = 0.193 m³/d} \\
\Rightarrow & \quad 0.193/6.57 = 0.029 \\
\Rightarrow & \quad 0.029 \times 5/7 = 0.021 = \frac{AgVT}{AgV_H}
\end{align*}
\]

The calculated \( \frac{AgVT}{AgV_H} \) differs by a factor of 2.6 from the former value (0.008).

2.) \( \frac{DFT}{DF_H} \)
DFT/DFH was calculated as shown in Table 7-4. We limit subsequent calculations to the pulmonary region, which results in a DFT/DFH factor of 0.2. Uncertainties in deposition come from hygroscopic growth for water-soluble particles but were not considered in this calculation.
Table 7-4: Example calculation of deposition fractions and DF_T/DF_H for cobalt metal (data from NTP (2014)), MPPD 3.04

<table>
<thead>
<tr>
<th></th>
<th>MPPD 3.04</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PU</td>
</tr>
<tr>
<td>DF_H</td>
<td>0.1147</td>
</tr>
<tr>
<td>DF_T</td>
<td>0.0227</td>
</tr>
<tr>
<td>DF_T/DF_H</td>
<td>0.20</td>
</tr>
</tbody>
</table>

3.) Normalisation

Because of the low water solubility of cobalt metal particles, in most former approaches, normalisation would have been to the total alveolar macrophage volume (NFH/NFT = 670 according to Kuempel et al. (2015) or NFH/NFT = 1110, according to Pauluhn (2011a) (Table 5-2). However, due to the significant lysosomal solubility (e.g., in the macrophages), the macrophage volume may not be decisive for normalisation. Hence, also normalisation to the lung surface should be considered (NFH/NFT = e.g., 150; Table 5-1). Within the 4 ratios approach we selected NFH/NFT= 600 ((150+1110)/2≈600) for example calculations below.

4.) Retention

The updated retention factor is derived from the elimination half-life from the lung in humans (300d) and the respective value for rats (60d), resulting in ELR_H/ERL_T=0.20. However, for substances with medium solubility in the German approach, a doubling of ELR_H/ERL_T is suggested. We take account of the lysosomal solubility by reducing the interspecies difference in elimination, i.e. by doubling from ELR_H/ERL_T from 0.20 to 0.4. However, as discussed in Sections 6.4 and 6.9, species effects in retention are expected, if the interstitium compartment is included. This is the case for cobalt metal, where interstitium effects were observed in the rat, although only at higher doses. Therefore, according to Section 7.6, an additional factor of 2 (IIF= 0.5) is suggested for both, the 4 ratios approach and the aggregate 3 ratios approach.

In the aggregate 3 ratios approach factors for normalisation and for retention are combined to a single NEIR of 300 (Section 7.4).

5.) Overall HEC

This example assessment provides a HEC for cobalt metal, based on the study by NTP (2014). If each single factor is quantified (4 ratios approach) this results in the following HEC:

HEC= 0.021 x 0.2 x 600 x 0.4 x 0.5 x cT = 0.5 x cT

If the aggregate 3 ratios approach of Section 7.4 is used,

HEC= 0.021 x 0.2 x 300 x 0.5 x cT = 0.63 x cT

With a LOAEC_rat of 1.25 mg/m³ these two calculations result in similar values of LOAEC_{HEC}= 0.63 (4 ratios approach) – 0.79 mg/m³ (3 ratios approach).
6.) Discussion

Again this example calculation provided \( \text{LAE}_{\text{HEC}}, \) which were similar to the \( \text{LOAEC}_{\text{rat}} \) in the animal study, because of a \( \text{HEC}/\text{CT} = 0.5 \) (4 ratios approach) and a very similar \( \text{HEC}/\text{CT} \) of 0.63 (aggregate 3 ratios approach). A small deposition fraction of only \( \approx 2 \% \) in the rat resulted in large interspecies difference in deposition (\( \text{DF}_T/\text{DF}_H = 0.2 \)). However, the factors used for normalisation and retention in the 4 ratios approach have been selected only as example quantification for the purposes of this calculation and are not agreed by expert evaluations.

7.8.4 HEC – calculation from mice data for particles with lysosomal solubility: cobalt metal

This example illustrates the use of MPPD version 3.04 in combination with further updates in HEC calculation discussed in the present report for a substance, which is poorly soluble in water (2.9 mg/Liter at 20°C) and in epithelial lining fluid (4.8 \% solubility for extra fine particles at pH 7.4), but soluble in lysosomal fluid (92.4 \% solubility for extra fine particles, at pH 4.5) (AGS, 2017). This example is performed with parameters for mice and can be compared to the example above (Section 7.8.3) with rats. The experimental data, as reported by NTP (2014) for cobalt metal were used for parameter specification. It is not intended to present an overall assessment on cobalt metal, but only to compare the HEC-transformed NOAEC in rats with the corresponding human NAEC.

**Input parameters:**

- **LOAEC (mice)** = 1.25 mg/m³
- **MMAD**: 1.8 µm
- **GSD**: 1.7
- **Density**: 8.81 g/cm³
- **Exposure**: 6h/d; 5d/w
- **Strain**: B6C3F1-mice
- **Body weight**: 47.6 grams (average, male, after 52 weeks, NTP (2014))

**MPPD – calculation of deposition: version 3.04**

**Results:**

1.) \( \text{AgV}_T/\text{AgV}_H \)

Breathing frequency: 243 (from MPPD-exposure for given body weight, B6C3F1-mice)

Tidal volume: 0.262 mL (Note that the tidal volume, as suggested by MPPD template, has been corrected according to the allometric formula provided in Section 3.4)


\[ 22920 \text{ mL/d} \]

\[ \text{Breathing volume} = 0.0229 \text{ m}^3/\text{d} \]

\[ \frac{0.0229}{6.57} = 0.0035 \]

\[ \frac{0.0035 \times 5}{7} = 0.0025 = \frac{\text{AgV}_T}{\text{AgV}_H} \]

The calculated \( \frac{\text{AgV}_T}{\text{AgV}_H} \) differs only marginally (0.0025 vs. 0.0037) from the one derived in Section 3.4.

2.) \( \text{DF}_T/\text{DF}_H \)

\( \text{DF}_T/\text{DF}_H \) was calculated as shown in Table 7-5. We limit subsequent calculations to the pulmonary region, which results in a \( \text{DF}_T/\text{DF}_H \)-factor of 0.39. There is some influence in the deposition calculation by the assumed breathing pattern (breathing frequency, tidal volume) calculated from allometric formula by MPPD based on the given body weight. Note, that the tidal volume, as suggested by MPPD template, has been corrected according to the allometric formula provided in Section 3.4.

Table 7-5: Example calculation of deposition fractions in the mouse lung and \( \text{DF}_T/\text{DF}_H \) for cobalt metal (data from NTP (2014)), MPPD 3.04

<table>
<thead>
<tr>
<th></th>
<th>PU</th>
<th>TB</th>
<th>PU+TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{DF}_H )</td>
<td>0.1147</td>
<td>0.0372</td>
<td>0.1519</td>
</tr>
<tr>
<td>( \text{DF}_T )</td>
<td>0.0448</td>
<td>0.0219</td>
<td>0.0667</td>
</tr>
<tr>
<td>( \text{DF}_T/\text{DF}_H )</td>
<td>0.39</td>
<td>0.59</td>
<td>0.44</td>
</tr>
</tbody>
</table>

3.) Normalisation

Because of the low water solubility of cobalt metal particles, in most former approaches, normalisation would have been to the total alveolar macrophage volume. However, because of an assumable high solubility in lysosomal fluid, macrophage loading is assumed to be limited and effects may arise from macrophage damage as well as from damages of epithelia of the lung cells. For rats, we calculated \( \text{NF}_H/\text{NFT} \), influenced by both, macrophage volume and lunge surface. Such a factor is not available for mice and should not be proposed without more qualified data. Therefore we provide \( \text{NF}_H/\text{NFT} \) for both options (option 1: total macrophage volume; option 2: lung epithelial surface), to show the range of results for comparison.

4.) Retention

The updated retention factor is derived from the elimination half-life from the lung in humans (300d) and the respective value for mice (60d), resulting in \( \text{ELR}_H/\text{ELR}_T=0.2 \). However, for substances with medium solubility in the German approach, a doubling of \( \text{ELR}_H/\text{ELR}_T \) is suggested. We take account of the lysosomal solubility by reducing the interspecies difference in elimination, i.e. by doubling from \( \text{ELR}_H/\text{ELR}_T \) from 0.2 to 0.4 (both options). However, as discussed in Sections 6.4 and 6.9, species effects in
retention are expected, if the interstitium compartment is included. This is the case for cobalt metal, where interstitium effects were observed in the mouse.

5.) Overall HEC

This example assessment provides a HEC for cobalt metal, based on the study by NTP (2014). This results in the following HEC (option 1: normalisation to the alveolar macrophage volume of \( \approx 13000 \) according to Stone et al. (1992); Section 5.6):

\[
HEC = 0.0025 \times 0.39 \times 13000 \times 0.4 \times 0.5 \times c_T = 2.54 \times c_T,
\]

changed to \( \text{HEC/c}_T = 1 \) (according to Section 7.7)

If option 2 (normalisation to the lung epithelial surface based on data from Hsieh et al. (1999) were regarded more appropriate, HEC will be calculated accordingly:

\[
HEC = 0.0025 \times 0.39 \times 723 \times 0.4 \times 0.5 \times c_T = 0.14 \times c_T
\]

\[
\text{LOAEC}_{\text{HEC}} = 1.25 \text{ mg/m}^3 \times 1 = 1.25 \text{ mg/m}^3 \text{ (option 1) vs. } 1.25 \times 0.14 \approx 0.2 \text{ mg/m}^3 \text{ (option 2)}
\]

For mice, the aggregate 3 ratios approach has not been developed due to overall uncertainties (Section 7.5).

6.) Discussion

This last example demonstrates that HEC calculations with mice (\( \text{LOAEC}_{\text{HEC}} = 0.2-1.25 \text{ mg/m}^3 \)) do not necessarily contradict HEC from rat (\( \text{LOAEC}_{\text{HEC}} = 0.63-0.79 \text{ mg/m}^3 \)) Section 7.8.3). However, a significantly larger range of potential HEC values is derived from mice data due to uncertainties in normalisation. This supports our conclusion that HEC calculations from mice data are not sufficiently validated to be useful in regulatory standard setting.

7.8.5 Conclusions from the examples

The examples calculated above demonstrate that HEC/c\(_T\) is always greater than 0.5, if rat data were the starting point. Even though there may be examples with smaller HEC values, such cases are rarely expected. For particles with low solubility and/or relevant contributions of the alveolar macrophages clearance to the MoA the values become even closer to 1. If, however, other equally defendable values were chosen to quantify normalisation and elimination species differences, the influence of HEC on the final result could be substantial.

Differences between the 4 ratios approach and the aggregate 3 ratios approach are rather small: this supports to apply the aggregate 3 ratios approach, as this calculation does not pretend to discriminate the various influences on HEC as precisely as it may be erroneously concluded from the 4 ratios approach.

Considering the limited influence and the high uncertainty of HEC and the elaborate calculations to perform such HEC assessments, it could also be decided to abstain from any HEC calculation, i.e., select HEC/c= 1, if only a moderate protection goal is
regarded to be sufficient. If, however, a higher protection goal is regarded to be adequate, even a simple “assessment factor” could serve to fulfil this requirement and the time-consuming aggregate 3 ratios approach could also be waived. This does not preclude the use of all the HEC elements for interspecies extrapolations in case of non-default assessments. If, for example, there are indications of relevant species differences because of contradicting human and animal data, this situation could be analysed using the complete steps of HEC.

The examples also demonstrated the considerably larger range of HEC results for mice data compared to the rat. This is in agreement with our general reluctance to support HEC calculations based on mice data, as, currently, the uncertainties may be too high and the number of qualified data for mice may not be sufficient.

7.9 Summary of uncertainties in the HEC approach

When deriving an occupational exposure limit (OEL) for particles, interspecies extrapolation from rodents to men is frequently necessary. Therefore, it is important to define human exposure levels, which are regarded equivalent to the exposure level of the starting point (the experimental animal inhalation study). Thus, HEC calculation is a major element of interspecies extrapolation for particle exposure. However, as described more closely in Sections 3 to 6, there are several relevant uncertainties to be acknowledged, which are summarized below:

- Quantification of **weighted breathing volumes** should consider strain-specific data, because body weight significantly influences the breathing volume. Uncertainties come from discrepant allometric scaling procedures (MPPD vs. OEHHA), which do not equally cover data from some relevant rat strains and from high variability in breathing volume.

- For **deposition**, some relevant uncertainties arise from potential particle solubility (particle growth for hygroscopic particles), from the reference deposition area (pulmonary only vs. pulmonary plus tracheobronchial sites), and from the inhomogeneity of deposition (hot spots). The different versions of MPPD software may lead to significant discrepancies in the calculated deposition fraction ratio. However, this uncertainty is mainly relevant at larger particle sizes, beyond the default applicability range of the HEC approach.

- There are major uncertainties from the interrelationship between particle solubility and mode of action (MoA) on the one side and the unit for **dose metrics and normalisation** on the other side. It is not always evident, whether the primary site of deposition is also the critical target, relevant for normalisation, or if the site of secondary reactions is the more adequate reference point for normalisation and dose metrics. Frequently, more than one single MoA may be relevant, each with different optimal normalisation unit and a different optimal dose metric. Even if the critical target cells (e.g. lung alveolar epithelium cells or alveolar macrophages) can be identified, there are some discrepancies to quantify the normalisation factor.

- Similar uncertainties are obvious for interspecies clearance and **elimination rate** differences, when the retained dose is the starting point. Earlier procedures only considered alveolar macrophage mucociliary clearance as relevant for
interspecies differences. However, translocation to the interstitium needs also to be considered as one of the significant elimination routes with potential interspecies differences. Similarly, earlier approaches assumed that there would be no significant species difference for highly soluble particles in elimination. However, different solubility in the various lung fluids may influence MoA, retention time, and migration to intracellular regions or extracellular effects. If, for example alveolar macrophages are involved in lysis or transport of particles, species differences can be expected. Highly soluble particles may be bound to proteins with consequences in MoA and clearance mechanism with subsequent species differences. Again, inhomogeneity in elimination time due to local hot spot accumulation is not adequately covered by the HEC-calculation procedure.

- Further uncertainties are linked to the default applicability range for default HEC calculations: the experimental animal study should be performed with particles sizes in the range of [0.5-2] µm MMAD. Therefore, there may be many assessments and data, where this default needs to be modified with no detailed generic guidance. Specifically, specific considerations apart from the standard HEC-calculation may be necessary for nano-sized particles or agglomerates. It needs further elaborations or, at least transparent discussion of additional recent data, to ensure that OELs calculated for the range of micro-sized particles are equally applicable to the nano-sized agglomerates below 0.5 µm MMAD-equivalent. Moreover, HEC-linked standard setting for particles refer to all respirable particle sizes, where significant differences can be observed between the experimental particle size distribution and the size distribution relevant in the human workplace exposure scenario. As particle size dependent deposition fraction and interspecies deposition fraction ratio are not proportional to the size specific dose response relationship for adverse effects in the animal study, and as animals are usually only studied at a single particle size distribution, this difference in exposure sizes contributes to overall uncertainty. Interspecies extrapolation from small particle sizes in the animal study is not always protective, if humans are exposed to larger respirable particles.

In conclusion, calculation of HEC is one element within the framework of standard setting for particle effects, covering potential toxicokinetic differences between species. However, as shown with the list above, there remain substantial uncertainties with the application of the HEC procedure, which need to be addressed when deriving OELs for particulate substances. Further, other elements of interspecies extrapolation such as potential differences in toxicodynamics, which are outside the scope of this report, should also be addressed.
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