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A Physiologically Based Pharmacokinetic Model for Ionic Silver and Silver Nanoparticles

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Supporting Information

A Physiologically Based Pharmacokinetic Model for

Ionic Silver and Silver Nanoparticles

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1. Model Parameters, Equations and Assumptions

1.1. Physiological Parameters

For bone marrow and intestines data was taken, if available, from red bone marrow and small intestines, respectively.

Table S1: Physiological parameters for weight and blood flow of organs in % of total body weight and % of cardiac output, respectively. Values for (Wistar) rats are taken from Brown et al\(^1\) and for humans from the reference adult man ICRP\(^2\).

<table>
<thead>
<tr>
<th>Organ</th>
<th>Weight [%]</th>
<th>Blood Flow [%]</th>
<th>Weight [%]</th>
<th>Blood Flow [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>19.00</td>
<td>5.80</td>
<td>4.36</td>
<td>5.00</td>
</tr>
<tr>
<td>Liver</td>
<td>3.66</td>
<td>18.30</td>
<td>2.47</td>
<td>25.50</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.73</td>
<td>14.10</td>
<td>0.42</td>
<td>19.00</td>
</tr>
<tr>
<td>Muscles</td>
<td>40.43</td>
<td>27.80</td>
<td>39.73</td>
<td>17.00</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.20</td>
<td>0.85(^a)</td>
<td>0.21</td>
<td>3.00</td>
</tr>
<tr>
<td>Heart</td>
<td>0.33</td>
<td>5.10</td>
<td>0.45</td>
<td>4.00</td>
</tr>
<tr>
<td>Brain</td>
<td>0.57</td>
<td>2.00</td>
<td>1.99</td>
<td>12.00</td>
</tr>
<tr>
<td>Lung</td>
<td>0.50</td>
<td>2.10</td>
<td>0.68</td>
<td>2.50</td>
</tr>
<tr>
<td>Testes</td>
<td>0.46(^e)</td>
<td>0.81(^b)</td>
<td>0.05(^d)</td>
<td>0.05</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>1.40</td>
<td>10.14(^e)</td>
<td>0.89</td>
<td>10.00</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>2.10</td>
<td>12.20</td>
<td>1.63</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Cardiac Output [L/min] | 0.095 | 6.50 |
Blood Amount [L]       | 0.022\(^d\) | 5.30 |
Body Weight [kg]       | 0.30\(^d\) | 73.00 |

\(^a\) Wilkinson et al\(^3\)
\(^b\) Saypol et al\(^4\)
\(^c\) Tarhan et al\(^5\)
\(^d\) Lankveld et al\(^6\)
\(^e\) Davies and Morris \(^7\)

Table S2: Glutathione (GSH) concentrations of organs in µmol per gram (\(c_{\text{organ,GSH}}\))

<table>
<thead>
<tr>
<th>Organ</th>
<th>GSH Concentration</th>
<th>Reference</th>
<th>GSH Concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>0.37±0.08</td>
<td>8</td>
<td>0.46±0.08</td>
<td>12</td>
</tr>
<tr>
<td>Liver</td>
<td>9.37±0.35(^a)</td>
<td>9</td>
<td>6.40±0.40</td>
<td>13</td>
</tr>
<tr>
<td>Kidneys</td>
<td>2.45±0.06(^a)</td>
<td>9</td>
<td>4.00±0.30</td>
<td>13</td>
</tr>
<tr>
<td>Muscles</td>
<td>0.73±0.07(^a)</td>
<td>9</td>
<td>1.80±0.08</td>
<td>14</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.12±0.04(^a)</td>
<td>9</td>
<td>2.12±0.04(^b)</td>
<td>9</td>
</tr>
<tr>
<td>Heart</td>
<td>2.12±0.21(^a)</td>
<td>9</td>
<td>1.20±0.20</td>
<td>13</td>
</tr>
<tr>
<td>Brain</td>
<td>1.64±0.01(^a)</td>
<td>9</td>
<td>1.40±0.30</td>
<td>13</td>
</tr>
<tr>
<td>Lung</td>
<td>2.04±0.01(^a)</td>
<td>9</td>
<td>2.04±0.01(^b)</td>
<td>9</td>
</tr>
<tr>
<td>Testes</td>
<td>3.41±0.20(^a)</td>
<td>10</td>
<td>3.41±0.20(^b)</td>
<td>10</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>1.78±0.24</td>
<td>8</td>
<td>1.78±0.24(^a)</td>
<td>8</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>2.20±0.22</td>
<td>11</td>
<td>0.48±0.20(^a)</td>
<td>15</td>
</tr>
</tbody>
</table>

Mean GSH Concentration | 1.22±0.10 | 1.87±0.11 |

\(^a\) Protein content according to Forbes et al\(^16\)
\(^b\) Rat values taken (no adequate human values found)
A PBPK model for ionic and nanosilver

Table S3: Classification of blood capillary and the physiologic upper limit of pore size according to Sarin\textsuperscript{17} as used for defining the capillary wall type groups

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Capillary type</th>
<th>Sub-Type</th>
<th>Physiologic upper limit of pore size</th>
<th>Representative tissue</th>
<th>Model Compartments</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT1</td>
<td>Non-sinusoidal non-fenestrated type</td>
<td>with tight junctions</td>
<td>&lt;1nm</td>
<td>Brain, Spinal Cord, Lymphoid Tissue Cortex</td>
<td>Brain, Muscles, Lung, Heart</td>
</tr>
<tr>
<td></td>
<td></td>
<td>with loose junctions</td>
<td>~5nm</td>
<td>Skin, Muscles, Lung, Cortical Bone, Intestinal Mesentery</td>
<td></td>
</tr>
<tr>
<td>CT2</td>
<td>Non-sinusoidal fenestrated type</td>
<td>diaphragmed fenestrae</td>
<td>6-12nm</td>
<td>Skin, Testis, Kidney Peritubular, Intestinal Mucosa</td>
<td>Skin\textsuperscript{a}, Testis, Kidneys, Intestines</td>
</tr>
<tr>
<td></td>
<td></td>
<td>open fenestrae</td>
<td>~15nm</td>
<td>Kidney Glomerulus</td>
<td></td>
</tr>
<tr>
<td>CT3</td>
<td>Sinusoidal reticuloendothelial type</td>
<td>open fenestrae</td>
<td>~180nm\textsuperscript{b}</td>
<td>Liver</td>
<td>Liver</td>
</tr>
<tr>
<td>CT4</td>
<td>Sinusoidal reticuloendothelial type</td>
<td>interendothelial junctions</td>
<td>~280nm\textsuperscript{b}</td>
<td>Liver</td>
<td>Liver</td>
</tr>
<tr>
<td>CT3</td>
<td>Sinusoidal non-reticuloendothelial type</td>
<td>terminal capillary ending</td>
<td>~5μm</td>
<td>Spleenic red pulp arterial blood capillary</td>
<td>Spleen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>interendothelial slits</td>
<td>—</td>
<td>Spleenic red pulp venous blood capillary</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Skin is partly in group CT1 and CT2 - For the calculations the more permeable group CT2 was chosen

\textsuperscript{b} Mouse and rat

1.2. Compound-dependent Parameters

Table S4: Ionic silver and nanosilver uptake constants

<table>
<thead>
<tr>
<th>Uptake Constants</th>
<th>Acronym</th>
<th>Organ</th>
<th>Parameter values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionic ([10^{-2}\text{ min}^{-1}])</td>
<td>(b_{\text{ionic}})</td>
<td>All</td>
<td>2.83\textsuperscript{a}</td>
</tr>
<tr>
<td>Transcapillary ([10^{-5}])</td>
<td>(b_{\text{nano, cap}})</td>
<td>CT1</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT2</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT3</td>
<td>2.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT4</td>
<td>21.60</td>
</tr>
<tr>
<td>MPS 1 ([10^{-5}\text{ nm}^{-2}])</td>
<td>(b_{\text{nano, MPS}_1})</td>
<td>Liver, Spleen</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lung</td>
<td>0.25</td>
</tr>
<tr>
<td>MPS 2 ([10^{-4}\text{ nm}^{-2}])</td>
<td>(b_{\text{nano, MPS}_2})</td>
<td>Liver, Spleen</td>
<td>1.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lung</td>
<td>0.50</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Decreased by a factor of 15 for the brain and testes
Table S5: Ionic silver distribution, metabolism and excretion rates for the rat and human PBPK model

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Rat</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ Release Rate $^{a,b}$ [$10^{-4}$ min$^{-1}$]</td>
<td>$k_{\text{organ_ionic_release}}$</td>
<td>5.78</td>
</tr>
<tr>
<td>Organ Storage Rate $^{a}$ [$10^{-6}$ min$^{-1}$]</td>
<td>$k_{\text{organ_ionic_storage}}$</td>
<td>0.95</td>
</tr>
<tr>
<td>Biliary Excretion Rate [$10^{-3}$ min$^{-1}$]</td>
<td>$k_{\text{ionic_biliary_excretion}}$</td>
<td>9.00</td>
</tr>
<tr>
<td>Urinary Excretion Rate [$10^{-3}$ min$^{-1}$]</td>
<td>$k_{\text{ionic_urinary_excretion}}$</td>
<td>0.13</td>
</tr>
</tbody>
</table>

$a$ The same release/storage rate was used for all organs
$^b$ Set to zero for the brain - see also Sano et al$^{18}$
$^c$ Increased by a factor of 14 for the skin

Table S6: Nanosilver distribution, metabolism and excretion rates for the rat and human PBPK model

<table>
<thead>
<tr>
<th>Nanosilver Model</th>
<th>Acronym</th>
<th>Rat</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ Release Rate $^{a,b}$ [$10^{-3}$ min$^{-1}$]</td>
<td>$k_{\text{organ_nano_release_cap}}$</td>
<td>6.50</td>
<td>1.90</td>
</tr>
<tr>
<td>Organ Release Rate MPS $^{a,d}$ [$10^{-3}$ min$^{-1}$]</td>
<td>$k_{\text{organ_nano_release_MPS}}$</td>
<td>0.72</td>
<td>0.21</td>
</tr>
<tr>
<td>Organ Storage Rate $^{a}$ [$10^{-6}$ min$^{-1}$]</td>
<td>$k_{\text{organ_nano_storage_cap}}$</td>
<td>0.95</td>
<td>0.95$^5$</td>
</tr>
<tr>
<td>Organ Storage Rate MPS $^{a,d}$ [$10^{-6}$ min$^{-1}$]</td>
<td>$k_{\text{organ_nano_storage_MPS}}$</td>
<td>9.46</td>
<td>9.46</td>
</tr>
<tr>
<td>Biliary Excretion Rate [$10^{-3}$ min$^{-1}$]</td>
<td>$k_{\text{nano_biliary_excretion}}$</td>
<td>9.00</td>
<td>3.54</td>
</tr>
<tr>
<td>Urinary Excretion Rate [$10^{-3}$ min$^{-1}$]</td>
<td>$k_{\text{nano_urinary_excretion}}$</td>
<td>0.13</td>
<td>0.39</td>
</tr>
</tbody>
</table>

$a$ The same release/storage rate was used for all organs
$^b$ Decreased by a factor of 4 for the brain and testes
$^c$ Increased by a factor of 14 for the skin
$^d$ Limited to the bone marrow and the MPS compartment of liver, spleen and lungs
- see also Locht et al$^{19}$ for a direct transformation of silver to silver sulphide by macrophages

Table S7: Intestinal absorption fractions

<table>
<thead>
<tr>
<th>Reference</th>
<th>Group</th>
<th>Fraction [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim et al.</td>
<td>low-dose group</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>middle-dose group</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>high-dose group</td>
<td>0.12</td>
</tr>
<tr>
<td>Loeschner et al.</td>
<td>nanoparticulate</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>ionic - with NP formation</td>
<td>3.25</td>
</tr>
<tr>
<td></td>
<td>ionic - without NP formation</td>
<td>3.85</td>
</tr>
</tbody>
</table>

Table S8: Distribution rates for time-dependent absorption into blood for the ICRP inhalation model

<table>
<thead>
<tr>
<th>Lung Distribution/Uptake Rates</th>
<th>Used for</th>
<th>Reference/Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>$s_d$ [$10^{-5}$ min$^{-1}$]</td>
<td>Figure 5A, 6C, 8 (Silver worker)</td>
<td>8.26</td>
</tr>
<tr>
<td>$s_l$ [$10^{-5}$ min$^{-1}$]</td>
<td>Figure 5B, 8 (Throat Spray)</td>
<td>1.15</td>
</tr>
<tr>
<td>$s_{ul}$ [$10^{-5}$ min$^{-1}$]</td>
<td>Figure 5C</td>
<td>1.40</td>
</tr>
<tr>
<td>$s_{pl}$ [$10^{-5}$ min$^{-1}$]</td>
<td>-</td>
<td>4.13</td>
</tr>
<tr>
<td>$s_{il}$ [$10^{-5}$ min$^{-1}$]</td>
<td>-</td>
<td>2.10</td>
</tr>
<tr>
<td>$c_l$ [min$^{-1}$]</td>
<td>-</td>
<td>0.63</td>
</tr>
<tr>
<td>$c_{ul}$ [$10^{-5}$ min$^{-1}$]</td>
<td>-</td>
<td>6.35$^5$</td>
</tr>
</tbody>
</table>

*a* Adapted for each case

*b* For the human model according to ICRP$^{20}$
### 1.3. Model Equations

Detailed information on the model equations can be found in the main text.

Table S9: Model equations of the ionic silver PBPK model (for rats and humans). The used model parameters and rates are listed in Table S1-S7. ("A" denotes the ionic silver amount in the corresponding compartment)

<table>
<thead>
<tr>
<th>Absorption</th>
<th>Summarized below in SI3 (Absorption Models)</th>
</tr>
</thead>
</table>
| Blood compartment: | \[
\frac{dA_{\text{blood,ionic}}}{dt} = \frac{\hat{m}_{\text{ab,der}} + \hat{m}_{\text{ab,ora}}}{1440} + \sum_{\text{Initial State compartments}} \left( S_p \cdot A_{\text{ICRP, lung, compartment}} \right) + \\
\sum_{\text{Organs*}} \left( k_{\text{organ,release}} \cdot A_{\text{organ,ionic}} \right) - \sum_{\text{Organs*}} \left( k_{\text{organ,up,ionic}} \cdot A_{\text{blood,ionic}} \right) - \\
k_{\text{ionic,biliary,excretion}} \cdot A_{\text{blood,ionic}} - k_{\text{ionic,urinary,excretion}} \cdot A_{\text{blood,ionic}} \right)
\] |
| Organ* compartment: | \[
\frac{dA_{\text{organ,ionic}}}{dt} = k_{\text{organ,up,ionic}} \cdot A_{\text{blood,ionic}} - k_{\text{organ,release}} \cdot A_{\text{organ,ionic}}
\] |
| Organ* uptake rate: | \[
k_{\text{organ,up,ionic}} = b_{\text{ionic}} \cdot \frac{m_{\text{organ, GSH}} \cdot c_{\text{organ, GSH}}}{m_{\text{B.W.}} \cdot c_{\text{body, GSH}}}
\] |

<table>
<thead>
<tr>
<th>Distribution</th>
<th></th>
</tr>
</thead>
</table>
| Organ* storage compartment: | \[
\frac{dA_{\text{organ,storage}}}{dt} = k_{\text{organ,storage}} \cdot A_{\text{organ,ionic}}
\] |
| Lung storage compartment: | \[
\frac{dA_{\text{lung,storage}}}{dt} = k_{\text{organ,storage}} \cdot A_{\text{lung,ionic}} + \sum_{\text{Transformed State compartments}} \left( S_t \cdot A_{\text{ICRP, lung, compartment}} \right)
\] |

<table>
<thead>
<tr>
<th>Metabolism</th>
<th></th>
</tr>
</thead>
</table>
| Biliary excretion: | \[
\frac{dA_{\text{biliary,excretion}}}{dt} = k_{\text{ionic,biliary,excretion}} \cdot A_{\text{blood,ionic}} \quad (+ \text{what is not absorbed in the GI tract after oral exposure})
\] |
| Urinary excretion: | \[
\frac{dA_{\text{urinary,excretion}}}{dt} = k_{\text{ionic,urinary,excretion}} \cdot A_{\text{blood,ionic}}
\] |

* Organ: bile (no storage), bone marrow, brain, heart, intestines, kidneys, liver, lung, muscles, skin, spleen and testes compartment
Table S10: Model equations of the nanosilver PBPK model (for rats and humans). The used model parameters and rates are listed in Table S1-S7. (“A” denotes the nanosilver amount in the corresponding compartment, “X” denotes the particles diameter – as we assumed: silver nanoparticles are discretely distributed in 1 nm steps from 1 to 150 nm, each nanometer representing a sub-compartment in each tissue and organ compartment (see also Figure S1). Thus, for the nanosilver PBPK model 151 compartment models are simulated in parallel (including the ionic silver PBPK model), each representing a discrete size of 1nm. Parameters and rates that are not highlighted by an “X” were assumed to be size-independent and have the same value for all particle sizes. Compartments that are not highlighted by an “X” take up all nanoparticle sizes in a common compartment, and also ionic silver (ie storage, feces and urine compartment))

<table>
<thead>
<tr>
<th>Absorption</th>
<th>Summarized below in SI3 (Absorption Models)</th>
</tr>
</thead>
</table>
| Blood compartment: | \[
\frac{dA_{\text{blood nano }X}}{dt} = \frac{\bar{m}_{\text{ab dep}} + \bar{m}_{\text{ab ora}}}{1440} + \sum_{\text{'Initial State' compartments}} \left( s_p \ast A_{\text{ICRP lung compartment }X} \right) + \sum_{\text{Organs}^{*}} \left( k_{\text{organ release cap}} \ast A_{\text{organ nano }X} \right) - \sum_{\text{Organs}^{*}} \left( k_{\text{organ up nano cap}} \ast A_{\text{blood nano }X} \right) + \sum_{\text{MPS}^{*}} \left( k_{\text{organ nano release MPS}} \ast A_{\text{MPS nano }X} \right) - \sum_{\text{MPS}^{*}} \left( k_{\text{organ up nano MPS }X} \ast A_{\text{blood nano }X} \right) - k_{\text{nano biliary excretion}} \ast A_{\text{blood nano }X} - k_{\text{nano urinary excretion}} \ast A_{\text{blood nano }X}
\]

| Distribution | |
| Organ* compartment: | \[
\frac{dA_{\text{organ nano }X}}{dt} = k_{\text{organ up nano cap}} \ast A_{\text{blood nano }X} - k_{\text{organ nano release cap}} \ast A_{\text{organ nano }X}
\]

| Organ* uptake rate: | \[k_{\text{organ up nano cap}} = b_{\text{nano cap}} \ast \frac{Q_{\text{organ blood}}}{V_{\text{blood}}}] |

| MPS* compartment: | \[
\frac{dA_{\text{MPS nano }X}}{dt} = k_{\text{organ up nano MPS }X} \ast A_{\text{blood nano }X} - k_{\text{organ nano release MPS}} \ast A_{\text{MPS nano }X}
\]

| MPS* uptake rate: | \[
k_{\text{organ up nano MPS }X} = \left[ b_{\text{nano MPS 1}} \ast d_{\text{particle diameter}}^2 + b_{\text{nano MPS 2}} \ast d_{\text{particle diameter}} \right] \ast \frac{Q_{\text{organ blood}}}{V_{\text{blood}}}
\]

Table continues on next page
### Metabolism

| Organ* storage compartment: | \[
\frac{dA_{\text{organ storage}}}{dt} = k_{\text{organ nano storage cap}} \times A_{\text{organ nano X}}
\] |
|----------------------------|------------------------------------------------------------------|
| MPS* storage compartment:  | \[
\frac{dA_{\text{MPS storage}}}{dt} = k_{\text{organ nano storage MPS}} \times A_{\text{MPS nano X}}
\] |
| Lung storage compartment:  | \[
\frac{dA_{\text{lung storage}}}{dt} = k_{\text{organ nano storage cap}} \times A_{\text{lung nano X}} + k_{\text{organ nano storage MPS}} \times A_{\text{lung MPS nano X}} + \sum_{\text{"Transformed State" compartments}} (S_t \times A_{\text{ICRP_lung compartment X}})
\] |
| Dissolution*: (all compartments, see also Figure S1) | \[
\frac{dA_{\text{all com nano X}}}{dt} = -k_{\text{diss model X}} \times A_{\text{all com nano X}}
\] |
| Excretion                  | \[
\frac{dA_{\text{biliary excretion}}}{dt} = k_{\text{nano biliary excretion}} \times A_{\text{blood nano X}} \quad (+ \text{what is not absorbed in the GI tract after oral exposure})
\] |
| Biliary excretion:         | \[
\frac{dA_{\text{urinary excretion}}}{dt} = k_{\text{nano urinary excretion}} \times A_{\text{blood nano X}}
\] |

* Organ: bone marrow (release and storage rate from MPS), brain, heart, intestines, kidneys, liver, lung, muscles, skin, spleen and testes compartment

* MPS (mononuclear phagocyte system): liver, lung and spleen compartment

* All compartments: organs, MPS and blood compartment. The dissolution per time was taken from literature. The dissolution rates of the specific diameters \(k_{\text{diss model X}}\) were calculated according to the dissolution per time (eg 5 nm/day).
1.4. Model Assumptions

Table S11: Summary of the assumptions made for the ionic silver and nanosilver PBPK model

<table>
<thead>
<tr>
<th>General</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Silver nanoparticles are discretely distributed in 1 nm steps from 1 to 150 nm, each nanometer representing a sub-compartment in each tissue and organ compartment.</td>
</tr>
<tr>
<td>● First-order kinetics is reasonably well describing the processes in vivo.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>● No transformation of ionic silver to nanosilver takes place during the uptake (through the skin, intestines and lung) to the systemic blood circulation (except nanoparticle formation in the GI tract, which is discussed in the text).</td>
</tr>
<tr>
<td>● If it is not specified in the toxicokinetic studies whether the nanoparticles were absorbed as particles or not, we assumed that the uptake was in particulate form.</td>
</tr>
<tr>
<td>● If information on the size distribution of released particles from consumer products was lacking it was assumed that they are in the nanoscale. When the size resolution was too low it was also assumed that all detected particles are in the nanoscale.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Distribution of nanoparticles in the blood stream is size-independent.</td>
</tr>
<tr>
<td>● Endocytosis processes are size-independent (except for the phagocytosis of particles by macrophages).</td>
</tr>
<tr>
<td>● The organ uptake of nanosilver via the capillary wall is proportional to the amount of silver that passes the capillary walls.</td>
</tr>
<tr>
<td>● Increase in the uptake efficiency with increasing particle diameter is identical for Kupffer cells and alveolar and splenic macrophages</td>
</tr>
</tbody>
</table>

Table continues on next page
Table S11 continued...

| Distribution | • Nanosilver that is neither distributed to organs (of our model) nor excreted is cleared from the blood by the bone marrow.  
• For nanosilver the transport efficiency through the capillary wall and the phagocytosis efficiency of macrophages are similar between rats and humans. |
| Metabolism | • The storage of ionic silver and nanosilver as silver sulfide (particles) is permanent and under normal exposure conditions no organ reaches saturation in a lifetime.  
• The dissolution time for silver nanoparticles that is reported in the literature (eg 5 nm/day) is comparable for all particles sizes (→ this kinetics is similar to the Higuchi model,\(^{21-22}\) which describes in vivo the dissolution of drug nanoparticles). |
| Excretion | • The formation of silver-GSH complexes takes place in all organs and further metabolisation of silver in the liver prior to excretion will not occur.  
• Biliary excretion of silver-GSH complexes is an endocytosis process that is comparable to the endocytosis of nanosilver. |
2. Particle Dissolution

In Figure S1 the overall PBPK model is depicted. The dissolution kinetics of the particles in the liver compartment is illustrated in Figure S2. Although the application domain of the model is from 15 to 150 nm, model simulations where particle dissolution was considered were carried out with sub-compartments from 1 to 150 nm. This was done in order to connect the ionic silver and nanosilver PBPK model properly.

As can be seen in Figure S2B, the error caused by particles below 15 nm is due the small mass negligible. However, this error increases in simulations where the particle size is close to 15nm. Therefore, simulations where dissolution was considered were solely carried out with particles larger than 60 nm (difference in mass of 60 nm and 15 nm particles is approximately two orders of magnitude).

Figure S1: Overall compartment model for silver including the dissolution and storage pathways of ionic silver and nanosilver. (*If the amount of dissolved silver corresponds with the amount of silver of the outermost 1 nm layer, the particle is transferred to the PBPK model of the next smallest size).
Figure S2: (A) Illustration of the dissolution kinetics using the example of the liver compartment. Simulation parameters: rat PBPK model; intravenous injection of 100 µg, 150 nm large silver nanoparticles; 30 nm/day dissolution; with MPS compartments. (B) The nanoparticulate silver level of the liver for the last four hours before the particle breakdown. Note: due to the large difference in mass between a 150 nm and a 1 nm large particle, a different scale was used for the y-axes than in graph A.
3. Calibration Results

2.1. Ionic Silver PBPK Model

The rat PBPK model for ionic silver was calibrated with in vivo toxicokinetic data from Klaassen\textsuperscript{23} that was obtained 2 hours after intravenous injection of an 100 µg/kg ionic silver solution. With the calibrated model, biodistributions were then also calculated for silver doses of 10, 30 and 300 µg/kg. As can be seen in Figure S3 the results are in good agreement with the data of Klaassen and there is an excellent linear correlation between the organ silver concentrations and the injected dose. In Figure S4 the blood and fecal silver levels are depicted. The blood silver levels reported by Klaassen are stabilizing around a silver concentration of around 300 µg/L. This behavior indicates that the blood itself has a silver storage capacity, which was not considered in the model. The temporary storage capacity can probably be attributed to the ability of several plasma proteins to bind silver ions.\textsuperscript{24}

2.2. Nanosilver PBPK Model

For nanosilver the transcapillary pathway and the mononuclear phagocyte system (MPS) of the rat PBPK model were calibrated separately with available toxicokinetic data from Lankveld et al.\textsuperscript{6} The calibration of the transcapillary pathway was limited to the tissue distribution after a single injection of 20 nm particles (see Figure S5A) and the time course of the blood silver concentration (Figure S6). For the MPS compartments the tissue distributions of all particle sizes and time periods were used (see Figure S5A-F). Although it was assumed that Kupffer cells and alveolar and splenic macrophages have the same quadratic particle uptake kinetics (see Equation 4, main text) the results of the MPS are fairly good. Only the lung silver levels after a single injection of 80 and 110 nm and the liver levels after 5 consecutive injections of 110 nm particles are considerably too high compared to the in vivo data. Although small uptake kinetic disparities exist, these results confirm that it is a valid approach to use a common quadratic particle uptake kinetic for the liver, spleen and lung.
Figure S3: PBPK rat model results compared with the experimental data by Klaassen. Values were obtained two hours after a single injection of an ionic solution containing (A) 10, (B) 30, (C) 100 or (D) 300 µg/kg silver. (*not evaluated by Klaassen, error bars represent standard deviations, n=5 or n=6).

Compartments which are limited to transcapillary uptake of nanosilver, may be compared with organ silver levels of Lankveld et al.⁶ that were not used to calibrate the model. As can be seen in Figure SSB-F, silver levels in the kidneys, heart and brain match well when compared to the in vivo data. The simulated silver levels in the testes fit also very well for day 1, but show too high levels after five days. This overestimation can be attributed to the conservative (small) release rate that was used for the testes and the brain. In conclusion, a size-dependent uptake via the capillary wall was not necessary to obtain good results in the organs that are not part of the MPS. In the PBPK model, even a slight decrease in the kidney silver level in the 80 and 110 nm group can be seen, which was reported by Lankveld et al.⁶ This decrease in the model is caused by an increased uptake of particles by the MPS (lung, liver, spleen), which especially leads to a slight decrease in the silver levels in the well-perfused kidneys.
To achieve mass balance, we assumed that non-recovered nanoparticles are cleared from the blood by the bone marrow. Hence, relatively high silver levels are predicted by the PBPK model. These non-recovered nanoparticles represent most of the silver, that neither can be attributed to the organ silver levels reported by Lankveld et al, nor to the biliary and urinary excretion of silver. The rest of this non-recovered nanosilver, even though much smaller quantities can be found in the intestines, skin and muscles. The uptake rates for these four compartments were determined with the data of the 20 nm particles. For the 80 nm and 110 nm particles the same uptake rates were used, which results for all particle sizes in the achievement of the mass balance. This indicates that there is a common clearance mechanism for all particle sizes, however for silver, the clearance of particles from the blood by the bone marrow still needs to be confirmed in vivo. In total, depending on the particle size, the silver in these four compartments represents between 45% and 71% of the injected silver after 5 consecutive injections at one day intervals.
Figure S4: Time course of the mean blood silver levels (A) and (B, C) fecal silver levels of Klaassen and the PBPK rat model. Values were obtained after a single injection of an ionic solution containing 100 µg/kg silver. (error bars represent standard deviations, n=3 to n=6).
Figure S5: PBPK rat model results of different particle size compared with the experimental data by Lankveld et al.\textsuperscript{6} Results show silver distribution one day after (A-C) a single injection and (D-F) 5 consecutive injections at intervals of one day. Each injection contained either (A, D) 23.8 (20.3 ± 1.9 nm), (B, E) 26.4 (79.8 ± 5.1 nm) or (C, F) 27.6 (112.6 ± 7.8 nm) µg silver. (*not evaluated by Lankveld et al, error bars represent standard deviations, n=3).
Figure S6: Time course of the mean blood silver levels over time (A) of Lankveld et al.\(^6\) and the PBPK rat model. Values were obtained after 5 consecutive injections at intervals of one day of a solution containing 23.8 \(\mu\)g (20.3 ± 1.9 nm) silver. First hour after injection at (B) day 1, (C) day 3 and (D) day 5 is shown enlarged. (error bars represent standard deviations, \(n=3\)).
4. Absorption Models

3.1. Dermal

The dermal uptake $\dot{m}_{ab,der}$ [mg/day] was calculated according to the following equation (based on ECHA$^{25}$):

$$\dot{m}_{ab,der} = \alpha_{Ag,der} \times A_{exp} \times f_{ab\text{per}}$$

Equation S1

where $\alpha_{Ag,der}$ [mg/cm$^2$/day] is the amount of released silver per cm$^2$ of T-shirt/crème/etc per day, $A_{exp}$ [cm$^2$] is the exposed skin area and $f_{ab\text{per}}$ [-] is the percutaneous absorption fraction.

The percutaneous absorption fraction is normally very low and values published in literature for ionic silver only represent the limit of detection and not the actual absorption.$^{26-27}$ Therefore, as a worst case assumption for healthy skin a 1% percutaneous absorption of ionic silver may be used. For nanosilver the available data is also very rare but it was shown by Larese et al.$^{28}$ that 0.0057% of 25±7.1 nm large silver particles can pass the human epidermis and dermis within one day. Although the authors could not determine whether the silver was absorbed in particulate or ionic form it is assumed for the model that particles can penetrate through the epidermis and reach the blood circulation. For both silver forms no increased storage in skin after dermal application and no lag time were considered. The percutaneous absorption fraction of 0.0057% was used for nanoparticles in the human PBPK model.

3.2. Oral

Oral uptake $\dot{m}_{ab,ora}$ [mg/day] was calculated according to the following equation (based on ECHA$^{25}$):

$$\dot{m}_{ab,ora} = c_{Ag,ora} \times m_{exp} \times f_{abora}$$

Equation S2

where $c_{Ag,ora}$ [mg/kg] is the concentration of silver in food and beverages, $m_{exp}$ [kg/day] is the amount of foods and beverages that is taken in per day and $f_{abora}$ [-] is the intestinal absorption fraction.
Different intestinal absorption fractions for ionic silver have been reported in literature. These values range from less than 0.5% in an in vitro intestinal epithelium coculture model,\textsuperscript{29} up to 1 to 10% in different animal models.\textsuperscript{30} For silver nanoparticles, similar values have been reported.\textsuperscript{29,31} Hence, if not mentioned otherwise, the same conservative intestinal absorption fraction of 4% for silver and nanosilver was used in the human model. In the PBPK models, (nano)silver that is not absorbed in the GI-tract to the systemic blood circulation is directly excreted to the feces. For both silver forms, no increased storage in the intestinal tract after dietary intake and no lag time were considered.

For ionic silver and nanosilver no liver specific metabolism process was incorporated into the PBPK models. Hence, a first-pass effect after oral intake can be neglected. Therefore, to keep the model structure simple, the ionic silver and nanosilver is directly added to the blood compartment.

3.3. Inhalation

For the human inhalation of particles the ICRP (International Commission on Radiological Protection) Human Respiratory Tract Model was used. For a detailed explanation of the model the reader is referred to Annals of the ICRP.\textsuperscript{20} Here we just present a very brief summary of the ICRP model: the lung is divided into five compartments, namely the extrathoracic region 1, the extrathoracic region 2, the bronchial region, the bronchiolar region and the alveolar-interstitial region. The deposition fraction of inhaled particles from 1 to 1000 nm among these five compartments is depicted in Figure S7. The mucociliary clearance rates were also taken from the ICRP model, and as recently shown, these rates are hardly influenced by the particle size and coating.\textsuperscript{32} Hence, in our model, the mucociliary clearance rates were used for all nanosilver sizes and coatings. For the time-dependent uptake of particles to the systemic blood circulation the ICRP model was adapted for our purposes (Figure S8). The distribution rates were estimated using in vivo kinetic data of rats, which is described below.
A PBPK model for ionic and nanosilver

For rats the inhalation model was simplified and, in contrast to the human model, only one compartment was used for the lung. With this approach it was avoided to implement size-dependent deposition and clearance processes between the lung compartments. Hence, all particles are deposited in a single lung compartment and mucociliary clearance is limited to a direct movement to the GI-tract. To estimate the distribution rates the time-dependent uptake model was not changed (Figure S8). The amount of inhaled silver $\dot{m}_{\text{inh}} \ [\text{mg/day}]$ was calculated according to the following formula:\textsuperscript{33}

$$\dot{m}_{\text{inh}} = c_{\text{Ag, inh}} \ast 0.001856 \ast m_{\text{b.w.}}^{0.92}$$ \text{ Equation S3}

where $c_{\text{Ag, inh}} \ [\text{mg/m}^3]$ is the silver concentration in air and $m_{\text{b.w.}} \ [\text{g}]$ is the body weight. In all cases it was assumed that 100% of the inhaled silver is deposited in the lungs.

The pulmonary absorption rates were estimated using experimental data by Takenaka et al.\textsuperscript{34} In Figure 3A (main text) and Figure S9, the lung and blood levels that were used as input data are highlighted with an asterisk (*). It was assumed that the particles are deposited in the lungs (“initial state”) and are then either taken up to the blood circulation or stabilized by proteins in the lung fluid (“transformed state”).\textsuperscript{35-36} Once stabilized the particles are transported to the GI-tract via mucociliary clearance or transformed to silver sulfide (“bound state” – see also metabolism section). The pulmonary absorption rate from “initial state” to the blood was determined for each exposure scenario separately. This adaptation became necessary because it can be seen in in vivo studies that the lung clears low amounts of silver more efficiently than higher ones.\textsuperscript{37} All distribution rates, and where they were used, are summarized in Table S8. For calculations with the human model, a pulmonary absorption rate was chosen, for which the exposure conditions were comparable to the in vivo conditions of the rat studies (that were used to determine the absorption rates).
A PBPK model for ionic and nanosilver

Figure S7: ICRP deposition model of the respiratory tract.

Figure S8: Compartment model for time-dependent absorption into blood adapted from the ICRP.20

Figure S9: Silver concentration of the lung of the PBPK model and of Takenaka et al.34 after exposure once for 6 hours to 133 μg/m³ of 14.6 ± 1.0 nm particles. Measurement points used as input data for the determination of the pulmonary distribution and absorption rates are highlighted with an asterisk. (error bars represent standard deviations, n=4).
5. Influence of Particle Dissolution

The evaluation of the influence of particle dissolution was carried out by using organ silver levels of rats that were orally exposed to 60 nm silver particles for 28 days.\textsuperscript{38} In this evaluation, dissolution is solely defined as Ag\textsuperscript{+} release from silver nanoparticles. In total we compared three different scenarios (Figure S10):

A. No in vivo dissolution takes place.

B. All particles dissolve at a rate of 7 nm per day (dissolution rate taken from Lee et al\textsuperscript{39}).

C. Only silver nanoparticles in the MPS (including the bone marrow) become completely dissolved (by macrophages). Therefore, the same MPS release rates were used as in the nanosilver PBPK model, but the released silver was added to the ionic blood compartment.

As can be seen in Figure S10, the best agreement with the in vivo data was obtained in the scenario where no dissolution of silver nanoparticles was assumed. Any kind of dissolution increases especially the silver level of the liver, which is rich in glutathione (GSH). Also, organs that take up rather low amounts of nanosilver show a considerable increase in the silver levels (i.e., heart, skin, muscles). Among these organs, the increase in the silver level can be seen very well in the example of the heart. Therefore, we included the heart silver levels to the results in Figure S10A-C. From the obtained results, we concluded that there is no significant dissolution of nanosilver in vivo. It should be noted that these results do not mean that the uptake of nanosilver by macrophages does not take place. However, the results are a good indication that silver nanoparticles that are taken up by macrophages are rather transformed directly into silver-sulfur nanocrystals than to silver ions. This transformation process was already shown in vitro for bulk silver by Locht et al.\textsuperscript{19,40}

Although this is only one example of the influence of dissolution on the organ silver levels, the comparison with other in vivo data showed very similar results (not shown here).
A PBPK model for ionic and nanosilver

Figure S10: Evaluation of the influence of dissolution on the organ silver levels. The PBPK model was compared to toxicokinetic data of rats after oral exposure to 300 mg/kg/day of 52.7–70.9 nm particles for 28 days (light grey – female rats, dark grey – male rats. *intestinal absorption fractions were fitted to match blood silver levels, °not evaluated by Kim et al, error bars represent standard deviations, n=10).

6. Sensitivity Analysis

We carried out a sensitivity analysis for the ionic silver and nanosilver PBPK model. However, both models are very comprehensive and many endpoints can be evaluated, e.g., various organs, rat and human, different exposure routes, or soluble and total silver concentrations. Since the structure of the ionic silver and nanosilver PBPK model is very simple and highly linear it was considered that a one-at-a-time sensitivity analysis (OAT-SA) is sufficient. Moreover, because we used the same linear organ uptake, release and storage kinetics the results of a sensitivity analysis are identical for nearly all organs. To include the exceptions that were implemented for some organs (i.e., blood-brain barrier and MPS) the OAT-SA was performed for the following three organs:

- Liver; Part of the MPS. High silver levels in the ionic silver and nanosilver PBPK model.
- Heart; Not part of the MPS. Low silver levels in the ionic silver and nanosilver PBPK model.
- Brain; Different uptake and release kinetics in both models due to the blood-brain-barrier.
A PBPK model for ionic and nanosilver

The endpoint that was evaluated for all three organs was the steady-state soluble silver concentration after oral intake of 90 µg silver per day. The results can be found in Figure S9A-S11A (ionic silver) and Figure S9B-S11B (nanosilver). In all cases the uptake and release rates of the organ were the most sensitive factors, followed by the biliary excretion (a short reminder: silver uptake is proportional to the GSH concentration, nanosilver uptake to the blood flow). The only non-linear kinetics of the model, namely the size-related uptake of nanoparticles of the MPS, plays a major role for organs of the MPS, which can be clearly seen at the very top of Figure S11B. All other parameters play practically no role. In general, the organ silver level was not affected by the change of a parameter of any other organ of the PBPK model (eg increasing the GSH concentration of the kidney by 1% had no influence on the soluble silver concentration of the liver). Therefore, the respective parameter was changed for all other organs of the model simultaneously (eg increasing the GSH concentrations of all organs by 1%, except the concentration of the liver).

In Figure S9C-S11C the influence of the particle dissolution is evaluated. It can be seen clearly that with an increasing dissolution rate, the organ silver levels converge to the levels that would be obtained by a dietary intake of 90 µg ionic silver per day. In solution, it was reported that silver nanoparticles dissolve at a rate of around 0.7 to 7 nm/day.\textsuperscript{39,41} Considering these dissolution rates, in the model, the silver concentration of the liver, heart and brain would increase/decrease in the range of around 40%, 700% and 5,000% compared to a scenario where particles do not dissolve, respectively. Especially the silver levels of the heart are a good indicator if the silver was distributed in ionic or nanoparticulate form. For the liver, the differences between the ionic silver and nanosilver levels are too small and the ionic silver in the brain accumulates to a much higher degree as nanosilver, which makes a distinction after short-term exposure much harder. However, in none of the studies that we used to validate the model could we find any evidence that would support a significant dissolution rate (see also Chapter “Influence of Particle Dissolution”). Therefore, in all calculations it was assumed that no significant dissolution takes place. It
should be noted that it cannot be excluded that by calibrating the PBPK model with the data of Lankveld et al\textsuperscript{6} some kind of dissolution was unintentionally included into the model or that the particles dissolve intracellular and are directly transformed to insoluble silver species within the cell.

Figure S11: Normalized sensitivity coefficients for model parameters using the steady-state soluble silver concentration of the human liver as endpoint of the sensitivity analysis. Exposure Scenario: 90 µg dietary silver intake per day. (A) Ionic silver PBPK model and (B) nanosilver PBPK model (with MPS, 80 nm particles). Bars indicate the change in the output produced by a 1% change of the parameter. (C) Influence of the dissolution rate on the steady-state soluble silver concentration of the human liver compared to no dissolution (dashed line shows the ionic steady-state soluble silver concentration of the same exposure scenario).
A PBPK model for ionic and nanosilver

Figure S12: Normalized sensitivity coefficients for model parameters using the steady-state soluble silver concentration of the human heart as endpoint of the sensitivity analysis. Exposure Scenario: 90 µg dietary silver intake per day. (A) Ionic silver PBPK model and (B) nanosilver PBPK model (with MPS, 80 nm particles). Bars indicate the change in the output produced by a 1% change of the parameter. (C) Influence of the dissolution rate on the steady-state soluble silver concentration of the human heart compared to no dissolution (dashed line shows the ionic steady-state soluble silver concentration of the same exposure scenario).

Figure S13: Normalized sensitivity coefficients for model parameters using the steady-state soluble silver concentration of the human brain as endpoint of the sensitivity analysis. Exposure Scenario: 90 µg dietary silver intake per day. (A) Ionic silver PBPK model and (B) nanosilver PBPK model (with MPS, 80 nm particles). Bars indicate the change in the output produced by a 1% change of the parameter. (C) Influence of the dissolution rate on the steady-state soluble silver concentration of the human brain compared to no dissolution (dashed line shows the ionic steady-state soluble silver concentration of the same exposure scenario).
7. References


