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## **Supplemental Material**

### **Size-Dependent Deposition, Translocation, and Microglial Activation of Inhaled Silver Nanoparticles in the Rodent Nose and Brain**

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## Relating experimental rodent inhalation of AgNP to human occupational exposure

To derive the experimental conditions of rodent inhalation exposure used in this study to the equivalent occupational exposure conditions for AgNP, the following assumptions and calculations were made:

1. AgNP deposition (mg) at a peak occupational area concentration (C) of 0.289 mg/m<sup>3</sup> with particles ranging 0.015-710.5 nm in aerodynamic diameter (Lee et al. 2012)

*Assumptions:*

- F = 44% = maximum fraction of AgNP deposited in the nasal region (Cheng and Swift 1995)
- T = 9600 min = monthly exposure period (60 min/h, 8 h/day, 5 days/wk, 4 wks)
- V = 15 L/min = minute ventilation for an adult human (Cheng and Swift 1995)

*Calculation of monthly AgNP deposition in the human nose:*

$$44\% \times \frac{0.289 \text{ mg Ag NP}}{\text{m}^3} \times \frac{\text{m}^3}{1000 \text{ L}} \times 9600 \text{ min} \times \frac{15 \text{ L}}{\text{min}} = 18.31 \text{ mg AgNP}$$

2. Monthly AgNP deposition normalized to surface area of the human nasal cavity (HNC)

*Assumption:*

- 181 cm<sup>2</sup> = SA of human nasal cavity (HNC) (Derelanko and Auletta 2014)

*Calculation:*

$$\frac{18.31 \text{ mg Ag NP}}{181 \text{ cm}^2 \text{ HNC}} = \frac{0.10 \text{ mg Ag NP}}{\text{cm}^2 \text{ HNC}}$$

### 3. Estimated rat exposure to AgNP in the current experiment

*Assumptions:*

- $F_{30} = 11\%$  = fraction of 30 nm AgNP deposited in the nasal region (Cheng and Swift 1995)
- $F_{110} = 4\%$  = fraction of 110 nm AgNP deposited in the nasal region (Cheng and Swift 1995)
- $C_{30} = 7.2 \pm 0.8 \text{ mg/m}^3$  = airborne concentration of silver in the 20 nm AgNP aerosol (Anderson et al. 2015)
- $C_{110} = 5.3 \pm 1.0 \text{ mg/m}^3$  = airborne concentration of silver in the 110 nm AgNP aerosol (Anderson et al. 2015)
- $T = 360 \text{ min}$  = experimental exposure period (60 min/h, 6 h/day)
- $V = 0.15 \text{ L/min}$  = minute ventilation for a 300 g rat
- $10.4 \text{ cm}^2$  = SA of rat nasal cavity (RNC) (Derelanko and Auletta 2014)

*Calculation of estimated silver deposition normalized to surface area of the rat nasal cavity after a single 6 h exposure to aerosolized AgNP with a 30 nm hydrodynamic diameter:*

$$11\% \times \frac{7.2 \text{ mg Ag NP}}{\text{m}^3} \times \frac{\text{m}^3}{1000 \text{ L}} \times 360 \text{ min} \times \frac{0.15 \text{ L}}{\text{min}} = 0.04 \text{ mg AgNP}$$

$$\frac{0.04 \text{ mg Ag NP}}{10.4 \text{ cm}^2 \text{ RNC}} = \frac{0.004 \text{ mg Ag NP}}{\text{cm}^2 \text{ RNC}}$$

*Calculation of estimated silver deposition normalized to surface area of the rat nasal cavity after a single 6 h exposure to aerosolized AgNP with a 110 nm diameter:*

$$4\% \times \frac{5.3 \text{ mg Ag NP}}{\text{m}^3} \times \frac{\text{m}^3}{1000 \text{ L}} \times 360 \text{ min} \times \frac{0.15 \text{ L}}{\text{min}} = 0.01 \text{ mg Ag NP}$$

$$\frac{0.01 \text{ mg Ag NP}}{10.4 \text{ cm}^2 \text{ RNC}} = \frac{0.001 \text{ mg Ag NP}}{\text{cm}^2 \text{ RNC}}$$

**Table S1.** Raw data on numbers of activated microglia to total microglia in control and AgNP-treated animals

<b>Time point</b>	<b>Activated Microglia</b>	<b>Resting Microglia</b>	<b>% of Activated Microglia</b>
Citrate Buffer	21	29	42
Citrate Buffer	33	17	66
Citrate Buffer	14	36	28
Citrate Buffer	19	31	38
Citrate Buffer	26	24	52
Citrate Buffer	30	20	60
20 nm AgNP T0	34	16	68
20 nm AgNP T0	36	14	72
20 nm AgNP T0	22	28	44
20 nm AgNP T1	39	11	78
20 nm AgNP T1	47	3	94
20 nm AgNP T1	31	19	62
20 nm AgNP T7	35	15	70
20 nm AgNP T7	33	17	66
20 nm AgNP T7	38	12	76
20 nm AgNP T21	32	18	64
20 nm AgNP T21	37	13	74
20 nm AgNP T21	25	25	50
20 nm AgNP T56	16	34	32
20 nm AgNP T56	34	16	68
20 nm AgNP T56	24	26	48
110 nm AgNP T0	38	12	76
110 nm AgNP T0	34	16	68
110 nm AgNP T0	32	18	64
110 nm AgNP T1	38	12	76
110 nm AgNP T1	26	24	52
110 nm AgNP T1	16	34	32
110 nm AgNP T7	31	19	62
110 nm AgNP T7	29	21	58
110 nm AgNP T7	19	31	38
110 nm AgNP T21	37	13	74
110 nm AgNP T21	32	18	64
110 nm AgNP T21	32	18	64
110 nm AgNP T56	32	18	64
110 nm AgNP T56	33	17	66

**Table S2.** Calculations of highest silver transport from the nose to the olfactory bulb at T0 for 20 nm and at T56 for 110 nm<sup>a</sup>

<b>Particle type</b>	<b>Silver deposition in olfactory region of nose (<math>\mu\text{g Ag/g tissue}</math>)</b>	<b>Percentage of silver translocation to olfactory bulb from olfactory region of nose (%)</b>
<b>20 nm AgNP</b>	$(0.03408 \mu\text{g Ag/g tissue in nose}) \times 0.2455 = \underline{0.0837}$	$(0.0132 \mu\text{g Ag/g tissue in OB}/0.0837 \mu\text{g Ag/g tissue in olfactory region of nose}) \times 100 = \underline{15.77\% \text{ at } T0}$
<b>110 nm AgNP</b>	$(0.4312 \mu\text{g Ag/g tissue in nose}) \times 0.35 = \underline{0.1509}$	$(0.0142 \mu\text{g Ag/g tissue in OB}/0.1509 \mu\text{g Ag/g tissue in olfactory region of nose}) \times 100 = \underline{9.41\% \text{ at } T56}$

<sup>a</sup>Calculations derived from Garcia and Kimbell (2009).

## References

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