# Repetitive Dosing of Fumed Silica Leads to Pro-Fibrogenic Effects Through Unique Structure-Activity Relationships and Biopersistence in the Lung

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**Table S1. Dosimetry calculations for fumed silica**

1. Calculated monthly amorphous silica in a silica manufacturing facility, where workers were exposed to 10.5 mg/m$^3$ precipitated amorphous silica.[1]

**Assumptions:**

· Ventilation rate of a healthy human adult: 20 [L/min]
· Deposition fraction: 30%
· Monthly exposure period: 8 [h/day], 5 [d/week], 4 weeks

**Calculation of monthly deposition:**

$$\frac{10.5\text{mg}}{\text{m}^3} \times \frac{20\text{L}}{\text{min}\cdot\text{person}} \times 30\% \times \frac{60\text{min}}{\text{hour}} \times \frac{8\text{hour}}{\text{day}} \times \frac{5\text{day}}{\text{week}} \times \frac{4\text{week}}{\text{month}} \times \frac{\text{m}^3}{1000\text{L}} = 604\ \text{mg/person}$$

**2. Monthly deposition level (mass/surface area) in a human worker**

**Assumptions:**

· Human alveolar surface area: 102 [m$^2$/person]

**Calculation:**

$$\frac{604\text{mg}}{\text{min}\cdot\text{person}} \times \frac{\text{person}}{102\text{m}^2} \times \frac{1000\mu\text{g}}{\text{mg}} = 5.921\ \text{mg/m}^2$$

**3. Comparable deposition level in a mouse receiving a one-time instillation**

Assumptions:

· Alveolar epithelium surface area of a mouse: 0.05 [m$^2$/mouse];
· Weight of a mouse: 25 [g]

**Calculation:**

$$\frac{5.921\text{mg}}{\text{m}^3\cdot\text{month}} \times \frac{0.05\ \text{m}^2}{\text{mouse}} \times \frac{\text{mouse}}{25\text{g}} \times \frac{1000\text{g}}{\text{kg}} = 11.98\ \text{mg/kg}$$

The chosen dose range of 6, 9 and 21 [mg/kg] in our study covers the calculated dose of 11.98 [mg/kg] per mouse, which is calculated based on a real-life exposure measurement of amorphous silica in a manufacturing facility.[1]

**Figure S1**

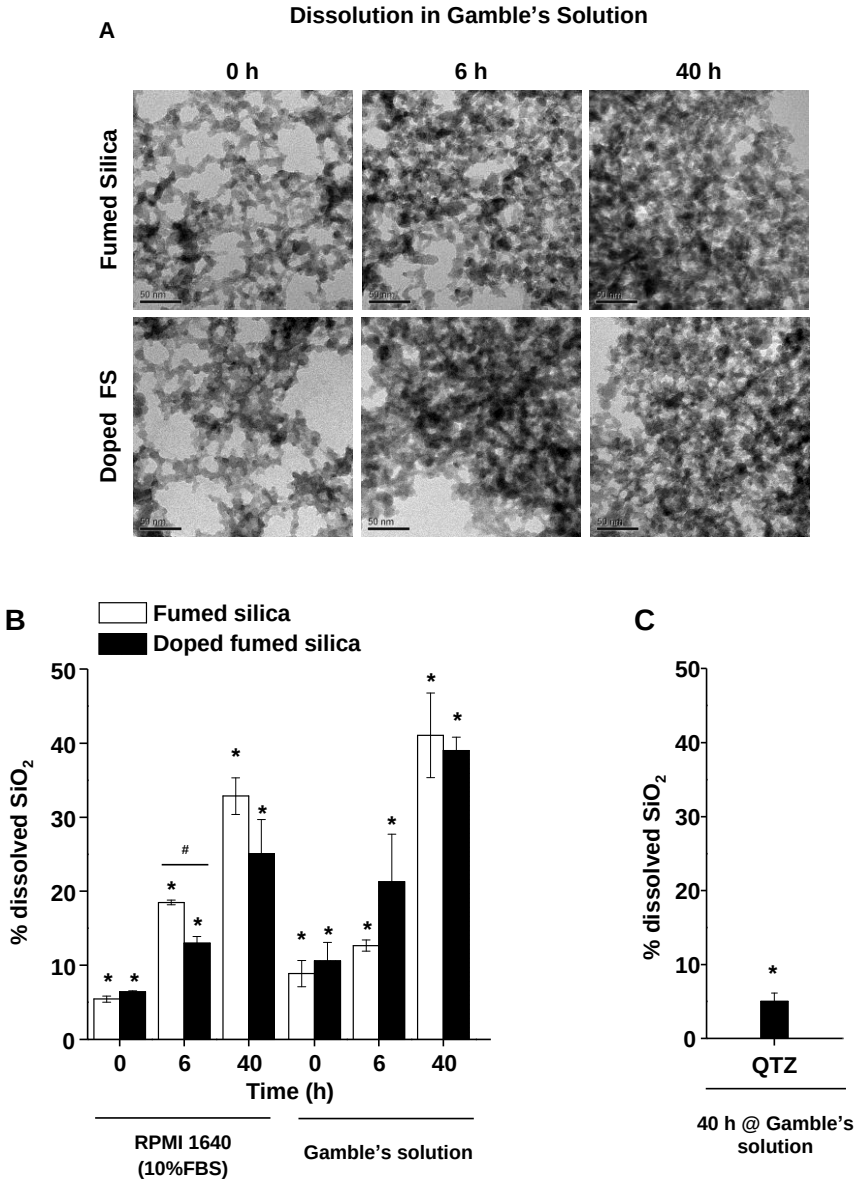


**Figure S1. Fumed silica dissolution in simulated biological fluids.** (A) TEM analysis showing silica dissolution in Gamble's Solution. 20 µL of the fumed silica stock solution (5 mg/mL) was added to 980 µL of exposure media before sonication. The particle suspension was incubated at 37 °C. Samples were taken at 0, 6, and 40 h, centrifuged at 15,000 rpm, and each pellet was washed and re-suspended in water for TEM analysis.

The scale bar is 50 nm. (B) ICP-OES analysis to study the time-dependent Si release from pristine and 7% Ti-doped fumed silica in various exposure media. 20 μL of each fumed silica stock solution (5 mg/mL) was added to 980 μL of exposure medium before sonication. The particle suspension was incubated at 37 °C. Samples were taken at 0, 6, and 40 h, centrifuged at 15,000 rpm, and each supernatant was collected for acid digestion and ICP-OES analysis. $^{*}p<0.05$ compared to control exposure media without particles. $^{\#}p<0.05$ compared to pristine fumed silica. (C) Dissolution of MIN-U-SIL, a natural form of α-quartz (QTZ), was assessed in Gamble solution for 40 hr at 37 °C. $^{*}p<0.05$ compared to control exposure media without particles.

**Figure S2**

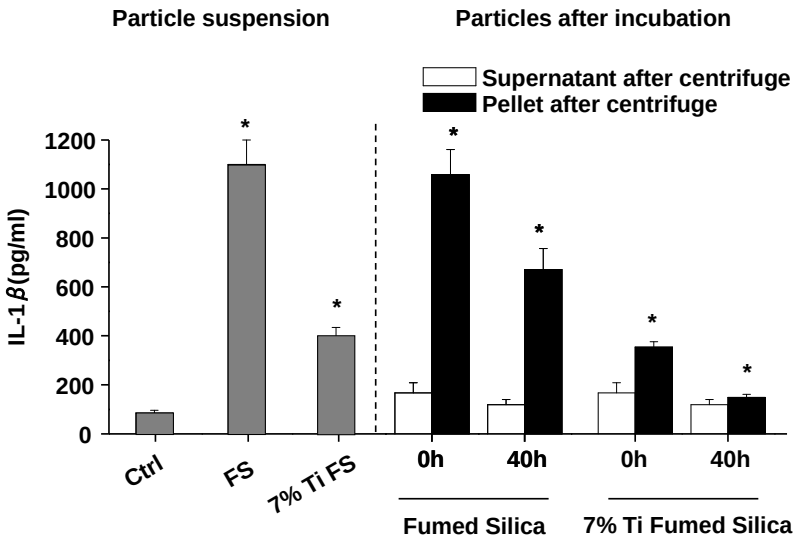


**Figure S2. The induction of IL-1β production by fumed silica was reduced by Ti-doping.** Fumed silica was added to Gamble's Solution at 100 μg/ml and the particle suspension was incubated at 37 °C. Samples were taken at 0 and 40 h, and centrifuged at 15,000 rpm. Supernatant and pellet were collected separately, and then exposed to THP-1 cells. $^{*}p<0.05$ compared to control cells without particle treatment.

**Figure S3**

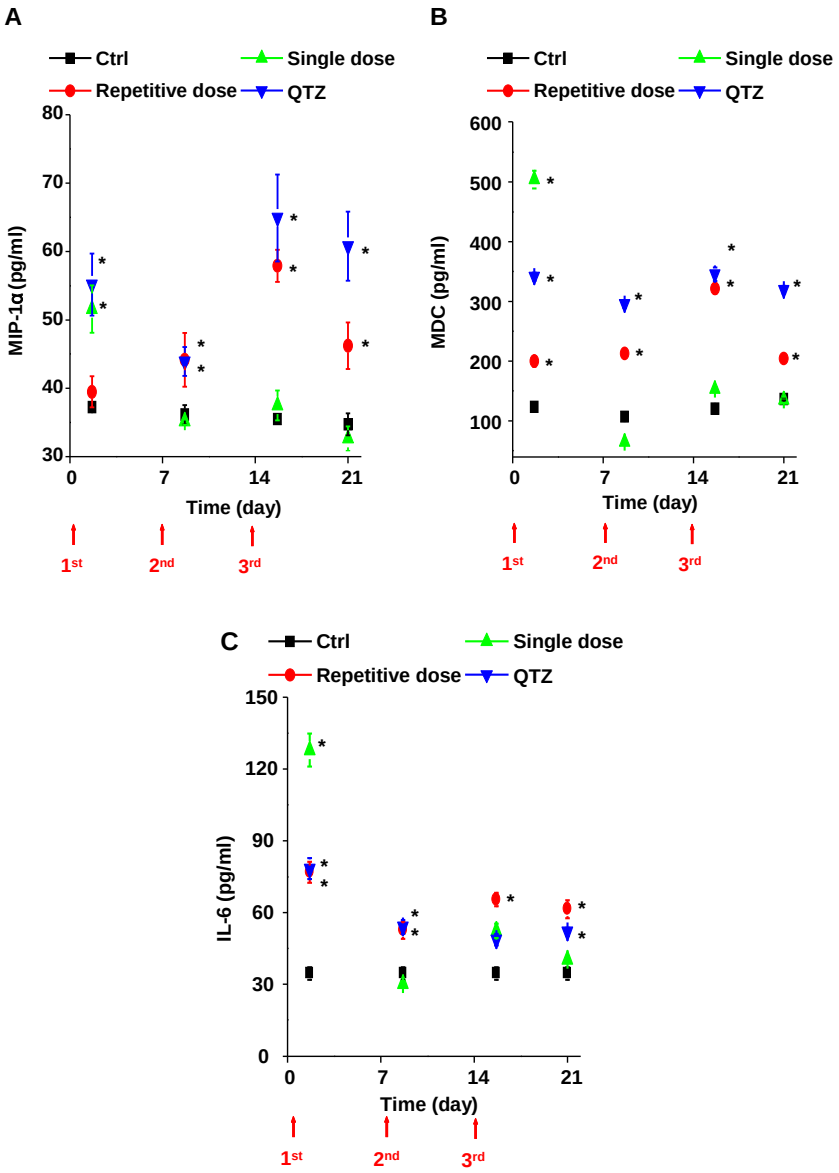


**Figure S3. Comparative analysis of the cytokine kinetics of single *vs.* repetitive dose fumed silica exposures.** C57BL/6 (n=6) mice were exposed to either a single dose of 21 mg/kg or 3 doses of 7 mg/kg fumed silica, one week apart, by oropharyngeal aspiration. On day 21, BAL fluid was collected to determine (A) MIP-1α, (B) MDC, and (C) IL-6 levels. $^{*}p<0.05$ compared to control mice without particle treatment.

**Figure S4**

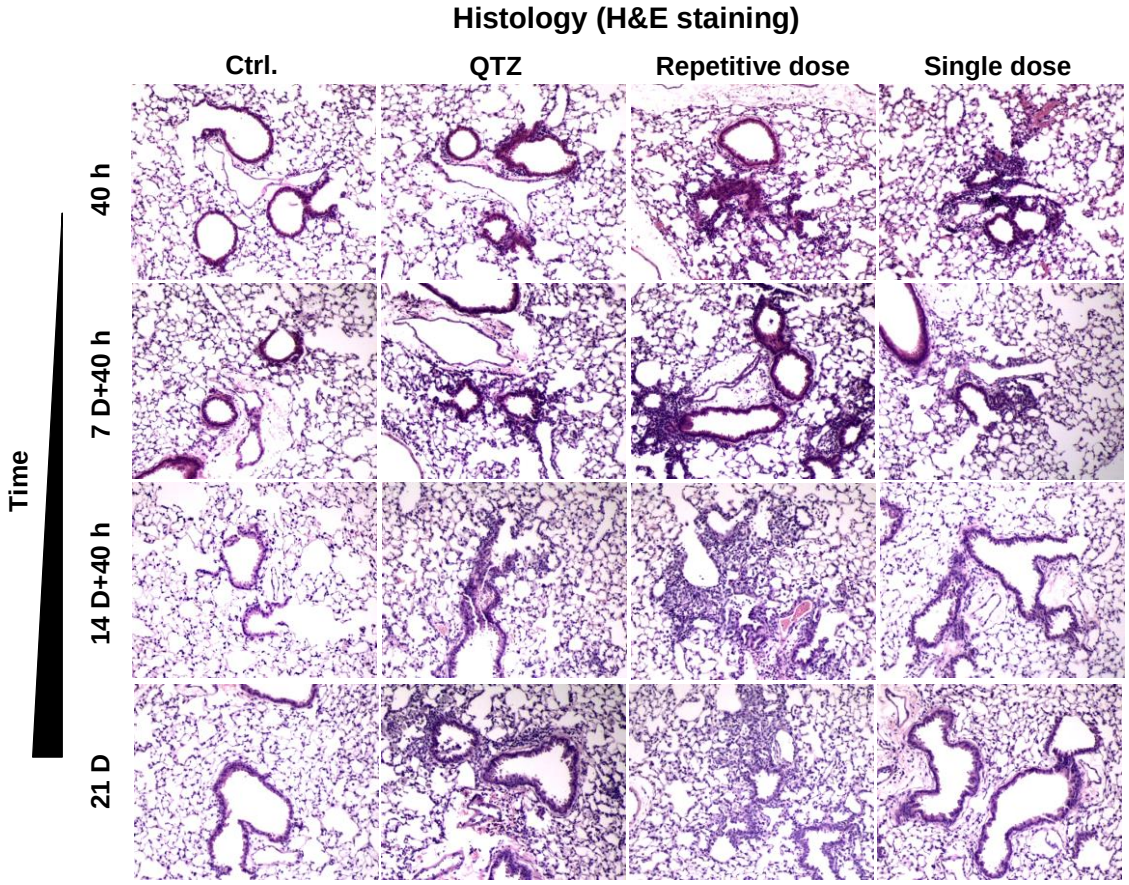


**Figure S4. Comparative analysis of lung inflammation kinetics induced by single *vs.* repetitive fumed silica exposures.** C57BL/6 (n=6) mice were exposed to either single dose of 21 mg/kg or 3 doses of 7 mg/kg fumed silica, one week apart, by oropharyngeal aspiration. On day 21, mice were sacrificed. H&E staining images showed the presence of focal inflammation in the lungs of animals treated with fumed silica.

**Figure S5**

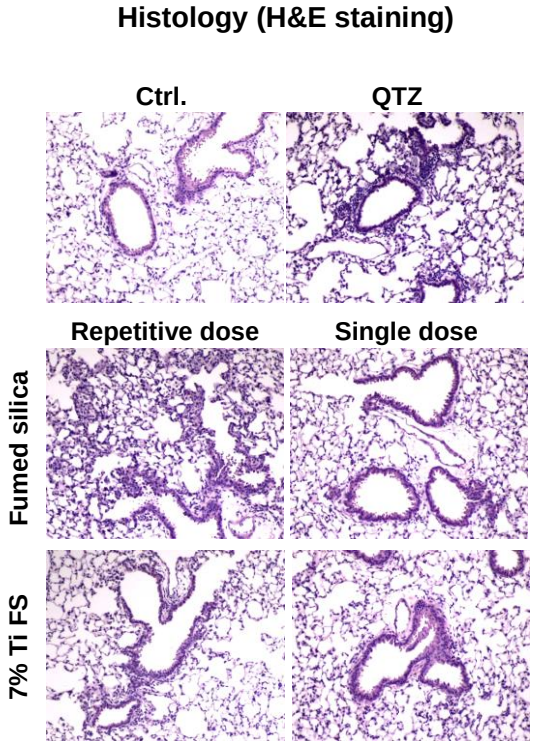


**Figure S5. Ti doping ameliorated the pro-fibrogenic effects of fumed silica during repetitive dosing.** C57BL/6 (n=6) mice were exposed to either a single dose of 21 mg/kg or 3 x 7 mg/kg pristine or 7% Ti-doped fumed silica, one week apart by oropharyngeal aspiration. On day 21, mice were sacrificed. H&E staining images show the presence of focal inflammation in the lungs of animals treated with fumed silica, and Ti doping reduce the inflammatory effects.

**References:**

1. Choudat, D.; Frisch, C.; Barrat, G.; el Kholti, A.; Conso, F., Occupational Exposure to Amorphous Silica Dust and Pulmonary Function. *Br. J. Ind. Med.* **1990**, 47, 763-766.