

Pulmonary effect of titanium dioxide and various particles by instillation to rats: in relation with surface area dose metrics.

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Background

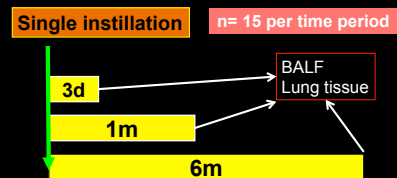
- Nanoparticles have specific optical and electric properties. Concerns about the biological effects of these nanoparticles being produced for use in industrial products have arisen since epidemiologic data have shown a correlation between airborne nanoparticles. There is a need for tests that simulate human exposure to nanoparticles to observe their health effects experimentally.
- Hubbs et al. reviewed that surface area to mass ratio increases as the size of particulates decreases, and the toxicity of particulates often but always, correlates with surface area more than mass. (Toxicologic pathology, 39: 301-324,2011)

Aim of this study

- We evaluated the pulmonary effect of nano-various titanium dioxide particles and other particles in relation with surface area.

Experimental design of Intratracheal Instillation

Animals : Wistar male rat
Materials : TiO₂ (**1.0 mg** / 0.4ml distilled water)
Observation time : 3d, 1m, 6 m

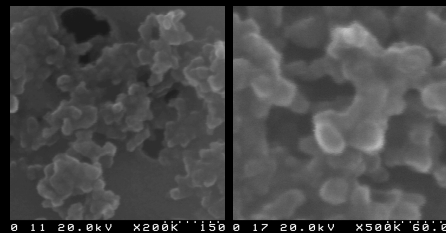


Titanium Dioxide (TiO₂)

name	company	Manufacturing process	BET surface area* (m ² /g)	Sauter mean diameter (nm)
P25	D	Gas phase reaction	53.8	28
P90	D	Gas phase reaction	102	15
anatase	T	Liquid phase deposition	102	14
rutile	T	Liquid phase deposition	102	14
amorphous	W	Liquid phase deposition	110	15

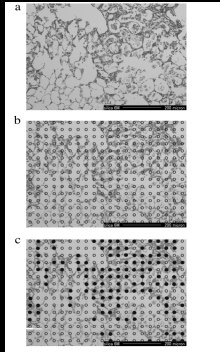
*determined by UBE Scientific Analysis Laboratory, Inc.
Specific gravity: rutile 4.2, anatase & amorphous 3.9

TiO₂ P90

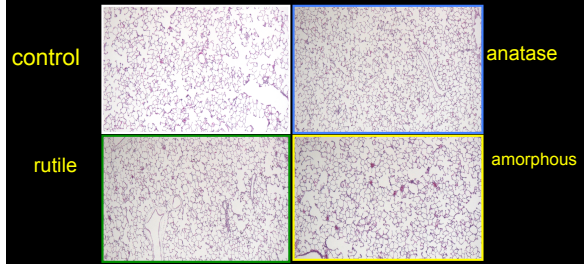


Evaluation of pulmonary Inflammation (Point Counting Method)

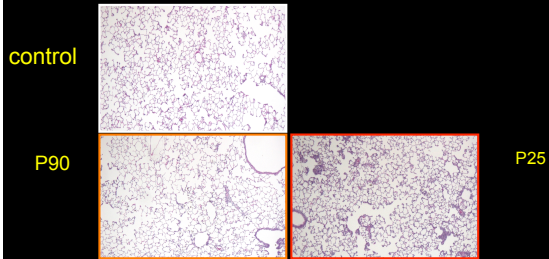
- a) Polarized light micrograph of a HE stained crystalline silica 6 months after intratracheal instillation. Magnification: x100
- b) Grids placed above a portion of the optical field, 300 points (20 x 15) are evenly provided.
- c) Points are directly positioned above each inflammation and counted as positive points



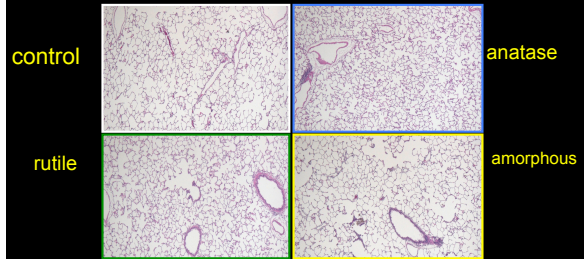
Lung tissue at 3 days after instillation



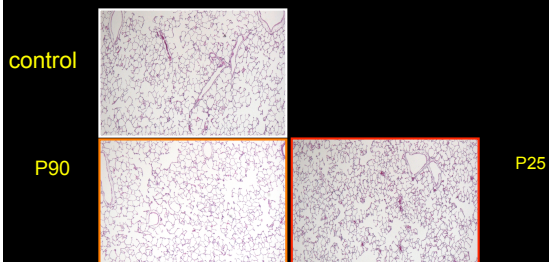
Lung tissue at 3 days after instillation



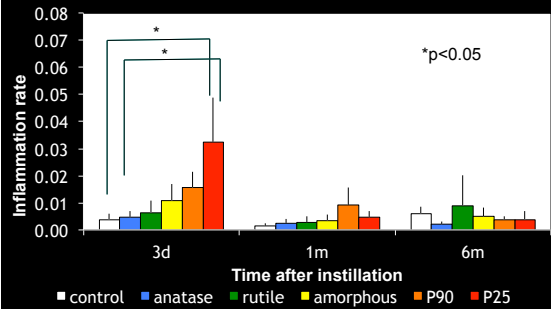
Lung tissue at 6 months after instillation



Lung tissue at 6 months after instillation



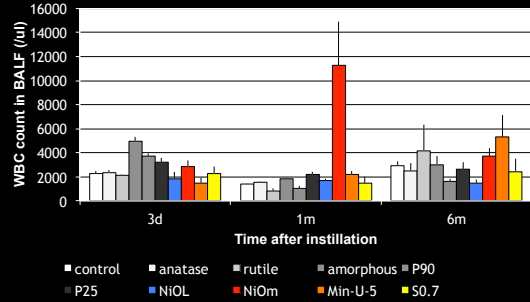
Inflammatory changes after intratracheal instillation in various TiO₂



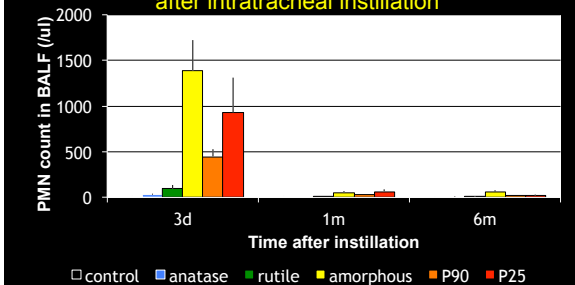
Summary of Pulmonary Histopathological Findings after Instillation of TiO₂

	anatase			rutile			amorphous			P90			P25		
	3d	1m	6m	3d	1m	6m	3d	1m	6m	3d	1m	6m	3d	1m	6m
macrophage accumulation in alveoli	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-
inflammatory cell infiltration	-	-	-	-	-	-	-	-	-	±	-	-	+	-	-
epithelial cell hyperplasia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

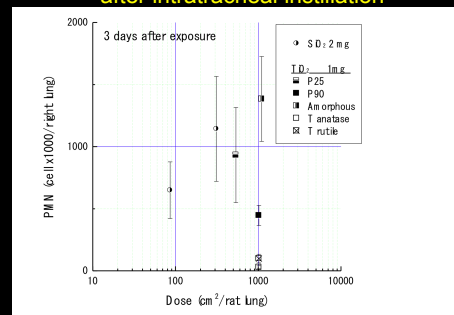
WBC count in BALF after intratracheal instillation



PMN count in BALF after intratracheal instillation



PMN count in BALF after intratracheal instillation



Summary of BALF Findings after Instillation of TiO₂

	anatase			rutile			amorphous			P90			P25		
	3d	1m	6m	3d	1m	6m	3d	1m	6m	3d	1m	6m	3d	1m	6m
PMN count in BALF	-	-	-	±	-	-	↑	-	-	↑	-	-	↑	-	-
Total cell count in BALF	-	-	-	-	-	-	↑	-	-	±	-	-	*	-	-
BET surface area* (m ² /g)	102	102	102	102	102	102	110	110	110	102	102	102	53.8	53.8	53.8
Sauter mean diameter (nm)	14	14	14	14	14	14	15	15	15	15	15	15	28	28	28

Results

■ Histological evaluation by point counting method

We instilled various type of TiO₂ (primary diameter less than 100nm) to rats. At 3days after instillation, significant inflammatory reactions such as macrophage infiltration or localized mild alveolitis were observed.

(P25>P90>amorphous>rutile>anatase).

However, at 1m and 6m after instillation, these inflammatory reaction recovered to the control level.

