

酸化チタン（ナノ粒子、アナターゼ型）の rasH2 マウス  
を用いた吸入による中期がん原性試験報告書

試験番号：0887

# APPENDICES

## APPENDICES

APPENDIX 1-1 IDENTITY OF TITANIUM DIOXIDE

APPENDIX 1-2 STABILITY OF TITANIUM DIOXIDE

APPENDIX 2 AEROSOL PARTICLE SIZE DISTRIBUTION ANALYSIS

APPENDIX 3 ENVIRONMENTAL CONDITIONS OF INHALATION CHAMBER

APPENDIX 4 METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY  
AND BIOCHEMISTRY

APPENDIX 1-1

IDENTITY OF TITANIUM DIOXIDE

## IDENTITY OF TITANIUM DIOXIDE

Test Substance : Titanium oxide (TAYCA CORPORATION)

Lot No. : 6545

## 1. Atomic Absorption Spectrophotometer

Instrument : Z-5010 Atomic Absorption Spectrophotometer (Hitachi, Ltd.)

Atomization : Graphite atomizer

Atomization temperature : 2700°C

Absorbance : 364.3 nm

Injection volume : 20 µL

---

	Titanium content (%)
Theoretical titanium value	60.0*
Titanium content in the titanium oxide	59.9

---

\*Theoretical titanium value was calculated by titanium element(48) and oxide element(16).

Result: The titanium content in the titanium oxide was consistent with theoretical value.

2. Conclusion: The test substance was identified as titanium oxide by atomic absorption spectrophotometer.

## APPENDIX 1-2

### STABILITY OF TITANIUM DIOXIDE

## STABILITY OF TITANIUM DIOXIDE

Test Substance : Titanium oxide (TAYCA CORPORATION)

Lot No. : 6545

## 1. Atomic Absorption Spectrophotometer

Instrument : Z-5010 Atomic Absorption Spectrophotometer (Hitachi, Ltd.)

Atomization : Graphite atomizer

Atomization temperature : 2700°C

Absorbance : 364.3 nm

Injection volume : 20 µL

Date Analyzed	Titanium content (%)
2017.06.05	59.9
2017.12.22	60.2

Result: The content of titanium in the test substance before and after use of the test substance was measured with an atomic absorption photometer. As a result, the content of titanium in the test substance before use almost agreed with the content of titanium in the test substance after use.

2. Conclusion: The test substance was stable for the period that the test substance had been used for the study.

## APPENDIX 2

### AEROSOL PARTICLE SIZE DISTRIBUTION ANALYSIS

## AEROSOL PARTICLE SIZE DISTRIBUTION ANALYSIS

### 1-WEEK ON ADMINISTRATION

Stage No. ( $\mu\text{m}$ )		2 mg/m <sup>3</sup> Flow : 10 $\ell$ /min      Time : 48min			8 mg/m <sup>3</sup> Flow : 10 $\ell$ /min      Time : 12min			32 mg/m <sup>3</sup> Flow : 10 $\ell$ /min      Time : 3min		
		Weight (mg)	Mass Fraction (%)	Cumulative Rate (%)	Weight (mg)	Mass Fraction (%)	Cumulative Rate (%)	Weight (mg)	Mass Fraction (%)	Cumulative Rate (%)
1	10	0.002	0.27	100.00	0.002	0.31	100.00	0.002	0.26	100.00
2	5.6	0.008	1.09	99.73	0.006	0.92	99.69	0.005	0.64	99.74
3	3.2	0.050	6.78	98.64	0.038	5.82	98.77	0.067	8.60	99.10
4	1.8	0.163	22.12	91.86	0.157	24.04	92.96	0.214	27.47	90.50
5	1.0	0.328	44.50	69.74	0.302	46.25	68.91	0.328	42.11	63.03
6	0.56	0.151	20.49	25.24	0.124	18.99	22.66	0.135	17.33	20.92
7	0.32	0.029	3.93	4.75	0.017	2.60	3.68	0.020	2.57	3.59
8	0.18	0.005	0.68	0.81	0.005	0.77	1.07	0.007	0.90	1.03
9	0.10	0.001	0.14	0.14	0.002	0.31	0.31	0.001	0.13	0.13
10	0.056	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
11	0.032	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
12	0.018	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
13	0.010	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
Final	~0.010	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
Total		0.737	100	-	0.653	100	-	0.779	100	-

## AEROSOL PARTICLE SIZE DISTRIBUTION ANALYSIS(CONTINUED)

## 13-WEEK ON ADMINISTRATION

Stage No. ( $\mu\text{m}$ )		2 mg/m <sup>3</sup> Flow : 10 $\ell$ /min      Time : 48min			8 mg/m <sup>3</sup> Flow : 10 $\ell$ /min      Time : 12min			32 mg/m <sup>3</sup> Flow : 10 $\ell$ /min      Time : 3min		
		Weight (mg)	Mass Fraction (%)	Cumulative Rate (%)	Weight (mg)	Mass Fraction (%)	Cumulative Rate (%)	Weight (mg)	Mass Fraction (%)	Cumulative Rate (%)
1	10	0.004	0.61	100.00	0.002	0.25	100.00	0.003	0.38	100.00
2	5.6	0.015	2.30	99.39	0.019	2.41	99.75	0.014	1.78	99.62
3	3.2	0.061	9.34	97.09	0.079	10.01	97.34	0.086	10.94	97.84
4	1.8	0.174	26.65	87.75	0.210	26.62	87.33	0.233	29.64	86.90
5	1.0	0.272	41.65	61.10	0.333	42.21	60.71	0.310	39.44	57.25
6	0.56	0.104	15.93	19.45	0.116	14.70	18.50	0.113	14.38	17.81
7	0.32	0.017	2.60	3.52	0.025	3.17	3.80	0.020	2.54	3.44
8	0.18	0.004	0.61	0.92	0.004	0.51	0.63	0.005	0.64	0.89
9	0.10	0.002	0.31	0.31	0.001	0.13	0.13	0.002	0.25	0.25
10	0.056	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
11	0.032	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
12	0.018	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
13	0.010	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
Final	~0.010	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
Total		0.653	100	-	0.789	100	-	0.786	100	-

## AEROSOL PARTICLE SIZE DISTRIBUTION ANALYSIS(CONTINUED)

25-WEEK ON ADMINISTRATION

Stage No. ( $\mu\text{m}$ )		2 mg/m <sup>3</sup> Flow : 10 $\ell$ /min      Time : 48min			8 mg/m <sup>3</sup> Flow : 10 $\ell$ /min      Time : 12min			32 mg/m <sup>3</sup> Flow : 10 $\ell$ /min      Time : 3min		
		Weight (mg)	Mass Fraction (%)	Cumulative Rate (%)	Weight (mg)	Mass Fraction (%)	Cumulative Rate (%)	Weight (mg)	Mass Fraction (%)	Cumulative Rate (%)
1	10	0.007	1.07	100.00	0.009	1.22	100.00	0.009	1.18	100.00
2	5.6	0.023	3.50	98.93	0.020	2.72	98.78	0.026	3.41	98.82
3	3.2	0.072	10.96	95.43	0.073	9.92	96.06	0.088	11.53	95.41
4	1.8	0.171	26.03	84.47	0.186	25.27	86.14	0.224	29.36	83.88
5	1.0	0.256	38.96	58.45	0.300	40.76	60.87	0.277	36.30	54.52
6	0.56	0.101	15.37	19.48	0.117	15.90	20.11	0.108	14.15	18.22
7	0.32	0.020	3.04	4.11	0.022	2.99	4.21	0.024	3.15	4.06
8	0.18	0.005	0.76	1.07	0.007	0.95	1.22	0.005	0.66	0.92
9	0.10	0.002	0.30	0.30	0.002	0.27	0.27	0.002	0.26	0.26
10	0.056	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
11	0.032	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
12	0.018	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
13	0.010	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
Final	~0.010	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00
Total		0.657	100	-	0.736	100	-	0.763	100	-

## APPENDIX 3

### ENVIRONMENTAL CONDITIONS OF INHALATION CHAMBER

## ENVIRONMENTAL CONDITIONS OF INHALATION CHAMBER

Group Name	Temperature (°C) Mean ± S.D.	Humidity (%) Mean ± S.D.	Ventilation Rate (L/min) Mean ± S.D.	Air Change (time/h) Mean
Control	23.0 ± 0.2	54.1 ± 1.4	414.2 ± 2.8	10.0
2 mg/m <sup>3</sup>	23.2 ± 0.3	56.7 ± 0.9	424.9 ± 5.2	10.3
8 mg/m <sup>3</sup>	23.1 ± 0.2	54.5 ± 0.8	421.3 ± 3.5	10.2
32 mg/m <sup>3</sup>	23.2 ± 0.3	56.2 ± 0.8	425.7 ± 3.7	10.3

## APPENDIX 4

### METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY

## METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY

Item	Method	Unit	Decimal place
<b>Hematology</b>			
Red blood cell (RBC)	Light scattering method <sup>1)</sup>	$\times 10^6/\mu\text{L}$	2
Hemoglobin(Hgb)	Cyanmethemoglobin method <sup>1)</sup>	g/dL	1
Hematocrit(Hct)	Calculated as $\text{RBC} \times \text{MCV}/10$ <sup>1)</sup>	%	1
Mean corpuscular volume(MCV)	Light scattering method <sup>1)</sup>	fL	1
Mean corpuscular hemoglobin(MCH)	Calculated as $\text{Hgb}/\text{RBC} \times 10$ <sup>1)</sup>	pg	1
Mean corpuscular hemoglobin concentration (MCHC)	Calculated as $\text{Hgb}/\text{Hct} \times 100$ <sup>1)</sup>	g/dL	1
Platelet	Light scattering method <sup>1)</sup>	$\times 10^3/\mu\text{L}$	0
Reticulocyte	Light scattering method <sup>1)</sup>	%	1
White blood cell(WBC)	Light scattering method <sup>1)</sup>	$\times 10^3/\mu\text{L}$	2
Differential WBC	Light scattering method <sup>1)</sup>	%	0
<b>Biochemistry</b>			
Total protein(TP)	Biuret method <sup>2)</sup>	g/dL	1
Albumin (Alb)	BCG method <sup>2)</sup>	g/dL	1
A/G ratio	Calculated as $\text{Alb}/(\text{TP} - \text{Alb})$ <sup>2)</sup>	—	1
T-bilirubin	BOD method <sup>2)</sup>	mg/dL	2
Glucose	GlcK·G-6-PDH method <sup>2)</sup>	mg/dL	0
T-cholesterol	CE·COD·POD method <sup>2)</sup>	mg/dL	0
Triglyceride	MGLP·GK·GPO·POD method <sup>2)</sup>	mg/dL	0
Phospholipid	PLD·ChOD·POD method <sup>2)</sup>	mg/dL	0
Aspartate aminotransferase (AST)	JSCC method <sup>2)</sup>	U/L	0
Alanine aminotransferase (ALT)	JSCC method <sup>2)</sup>	U/L	0
Lactate dehydrogenase (LDH)	JSCC method <sup>2)</sup>	U/L	0
Alkaline phosphatase (ALP)	JSCC method <sup>2)</sup>	U/L	0
$\gamma$ -Glutamyl transpeptidase ( $\gamma$ -GTP)	JSCC method <sup>2)</sup>	U/L	1
Creatine kinase (CK)	JSCC method <sup>2)</sup>	U/L	0
Urea nitrogen	Urease·GLDH method <sup>2)</sup>	mg/dL	1
Sodium	Ion selective electrode method <sup>2)</sup>	mEq/L	0
Potassium	Ion selective electrode method <sup>2)</sup>	mEq/L	1
Chloride	Ion selective electrode method <sup>2)</sup>	mEq/L	0
Calcium	OCPC method <sup>2)</sup>	mg/dL	1
Inorganic phosphorus	PNP·XOD·POD method <sup>2)</sup>	mg/dL	1

1) Automatic blood cell analyzer (ADVIA120 : Siemens Healthcare Diagnostics Inc.)

2) Automatic analyzer (Hitachi 7080 : Hitachi,Ltd.)