複層カーボンナノチューブ（MWCNT）のラットを用いた
吸入による13週間毒性試験報告書

試験番号：0780

APPENDICES

APPENDICES

APPENDIX 1

AEROSOL PARTICLE SIZE DISTRIBUTION ANALYSIS

Aerosol Particle Size Distribution Analysis (1st week)

[0.2 mg/m^3]

Sampling time: 360 min, Flow rate: 10 L/min

Stage No. (Cut-points)	Collection weight (mg)	Collection weight ratio (%)	Cumulative frequency (%)
1 (10.0 μm)	0.044	9.2	100.0
2 (5.6 μm)	0.141	29.7	90.8
3 (3.2 μm)	0.128	26.8	61.1
4 (1.8 μm)	0.081	17.0	34.3
5 (1.0 μm)	0.041	8.6	17.3
6 (0.56 μm)	0.023	4.8	8.7
7 (0.32 μm)	0.011	2.3	3.9
8 (0.18 μm)	0.005	1.0	1.6
9 (0.10 μm)	0.002	0.4	0.6
10 (0.056 μm)	0.001	0.2	0.2
11 (0.032 μm)	0.000	0.0	0.0
12 (0.018 μm)	0.000	0.0	0.0
13 (0.010 μm)	0.000	0.0	0.0
final (～0.010 μm)	0.000	0.0	0.0
Total	0.477	100.0	—

Aerosol Particle Size Distribution Analysis (6th week)

[0.2 mg/m^3]

Sampling time: 360 min, Flow rate: 10 L/min

Stage No. (Cut-points)	Collection weight (mg)	Collection weight ratio (%)	Cumulative frequency (%)
1 (10.0 μm)	0.053	8.8	100.0
2 (5.6 μm)	0.190	31.7	91.2
3 (3.2 μm)	0.139	23.2	59.5
4 (1.8 μm)	0.101	16.8	36.3
5 (1.0 μm)	0.059	9.8	19.5
6 (0.56 μm)	0.034	5.7	9.7
7 (0.32 μm)	0.012	2.0	4.0
8 (0.18 μm)	0.006	1.0	2.0
9 (0.10 μm)	0.003	0.5	1.0
10 (0.056 μm)	0.002	0.3	0.5
11 (0.032 μm)	0.001	0.2	0.2
12 (0.018 μm)	0.000	0.0	0.0
13 (0.010 μm)	0.000	0.0	0.0
final (～0.010 μm)	0.000	0.0	0.0
Total	0.600	100.0	—

Aerosol Particle Size Distribution Analysis (13th week)

[0.2 mg/m^3]

Sampling time: 360 min, Flow rate: 10 L/min

Stage No. (Cut-points)	Collection weight (mg)	Collection weight ratio (%)	Cumulative frequency (%)
1 (10.0 μm)	0.023	4.2	100.0
2 (5.6 μm)	0.181	33.2	95.8
3 (3.2 μm)	0.136	24.9	62.6
4 (1.8 μm)	0.094	17.2	37.7
5 (1.0 μm)	0.055	10.1	20.5
6 (0.56 μm)	0.032	5.9	10.4
7 (0.32 μm)	0.011	2.0	4.5
8 (0.18 μm)	0.006	1.1	2.5
9 (0.10 μm)	0.003	0.5	1.4
10 (0.056 μm)	0.003	0.5	0.9
11 (0.032 μm)	0.002	0.4	0.4
12 (0.018 μm)	0.000	0.0	0.0
13 (0.010 μm)	0.000	0.0	0.0
final (～0.010 μm)	0.000	0.0	0.0
Total	0.546	100.0	—

Aerosol Particle Size Distribution Analysis (1st week)

[1 mg/m^3]

Sampling time: 120 min, Flow rate: 10 L/min

Stage No. (Cut-points)	Collection weight (mg)	Collection weight ratio (%)	Cumulative frequency (%)
1 (10.0 μm)	0.077	7.8	100.0
2 (5.6 μm)	0.311	31.7	92.2
3 (3.2 μm)	0.261	26.6	60.5
4 (1.8 μm)	0.162	16.5	33.9
5 (1.0 μm)	0.085	8.6	17.4
6 (0.56 μm)	0.053	5.4	8.8
7 (0.32 μm)	0.019	1.9	3.4
8 (0.18 μm)	0.008	0.8	1.5
9 (0.10 μm)	0.005	0.5	0.7
10 (0.056 μm)	0.002	0.2	0.2
11 (0.032 μm)	0.000	0.0	0.0
12 (0.018 μm)	0.000	0.0	0.0
13 (0.010 μm)	0.000	0.0	0.0
final (～0.010 μm)	0.000	0.0	0.0
Total	0.983	100.0	—

Aerosol Particle Size Distribution Analysis (6th week)

[1 mg/m^3]

Sampling time: 120 min, Flow rate: 10 L/min

Stage No. (Cut-points)	Collection weight (mg)	Collection weight ratio (%)	Cumulative frequency (%)
1 (10.0 μm)	0.084	9.8	100.0
2 (5.6 μm)	0.268	31.5	90.2
3 (3.2 μm)	0.201	23.6	58.7
4 (1.8 μm)	0.139	16.3	35.1
5 (1.0 μm)	0.082	9.6	18.8
6 (0.56 μm)	0.047	5.5	9.2
7 (0.32 μm)	0.017	2.0	3.7
8 (0.18 μm)	0.008	0.9	1.7
9 (0.10 μm)	0.005	0.6	0.8
10 (0.056 μm)	0.002	0.2	0.2
11 (0.032 μm)	0.000	0.0	0.0
12 (0.018 μm)	0.000	0.0	0.0
13 (0.010 μm)	0.000	0.0	0.0
final (～0.010 μm)	0.000	0.0	0.0
Total	0.853	100.0	—

Aerosol Particle Size Distribution Analysis (13th week)

[1 mg/m^3]

Sampling time: 120 min, Flow rate: 10 L/min

Stage No. (Cut-points)	Collection weight (mg)	Collection weight ratio (%)	Cumulative frequency (%)
1 (10.0 μm)	0.033	3.9	100.0
2 (5.6 μm)	0.288	33.9	96.1
3 (3.2 μm)	0.256	30.2	62.2
4 (1.8 μm)	0.149	17.6	32.0
5 (1.0 μm)	0.051	6.0	14.4
6 (0.56 μm)	0.031	3.7	8.4
7 (0.32 μm)	0.022	2.6	4.7
8 (0.18 μm)	0.011	1.3	2.1
9 (0.10 μm)	0.003	0.4	0.8
10 (0.056 μm)	0.002	0.2	0.4
11 (0.032 μm)	0.002	0.2	0.2
12 (0.018 μm)	0.000	0.0	0.0
13 (0.010 μm)	0.000	0.0	0.0
final (～0.010 μm)	0.000	0.0	0.0
Total	0.848	100.0	—

Aerosol Particle Size Distribution Analysis (1st week)

[5 mg/m^3]

Sampling time: 30 min, Flow rate: 10 L/min

Stage No. (Cut-points)	Collection weight (mg)	Collection weight ratio (%)	Cumulative frequency (%)
1 (10.0 μm)	0.064	4.2	100.0
2 (5.6 μm)	0.400	26.1	95.8
3 (3.2 μm)	0.516	33.6	69.7
4 (1.8 μm)	0.316	20.6	36.1
5 (1.0 μm)	0.150	9.8	15.5
6 (0.56 μm)	0.052	3.4	5.7
7 (0.32 μm)	0.022	1.4	2.3
8 (0.18 μm)	0.008	0.5	0.9
9 (0.10 μm)	0.006	0.4	0.4
10 (0.056 μm)	0.000	0.0	0.0
11 (0.032 μm)	0.000	0.0	0.0
12 (0.018 μm)	0.000	0.0	0.0
13 (0.010 μm)	0.000	0.0	0.0
final (～0.010 μm)	0.000	0.0	0.0
Total	1.534	100.0	—

Aerosol Particle Size Distribution Analysis (6th week)

[5 mg/m^3]

Sampling time: 30 min, Flow rate: 10 L/min

Stage No. (Cut-points)	Collection weight (mg)	Collection weight ratio (%)	Cumulative frequency (%)
1 (10.0 μm)	0.102	8.7	100.0
2 (5.6 μm)	0.375	31.7	91.3
3 (3.2 μm)	0.281	23.9	59.6
4 (1.8 μm)	0.195	16.6	35.7
5 (1.0 μm)	0.114	9.7	19.1
6 (0.56 μm)	0.065	5.5	9.4
7 (0.32 μm)	0.023	2.0	3.9
8 (0.18 μm)	0.011	0.9	1.9
9 (0.10 μm)	0.006	0.5	1.0
10 (0.056 μm)	0.004	0.3	0.5
11 (0.032 μm)	0.002	0.2	0.2
12 (0.018 μm)	0.000	0.0	0.0
13 (0.010 μm)	0.000	0.0	0.0
final (～0.010 μm)	0.000	0.0	0.0
Total	1.178	100.0	—

Aerosol Particle Size Distribution Analysis (13th week)

[5 mg/m^3]

Sampling time: 30 min, Flow rate: 10 L/min

Stage No. (Cut-points)	Collection weight (mg)	Collection weight ratio (%)	Cumulative frequency (%)
1 (10.0 μm)	0.109	8.8	100.0
2 (5.6 μm)	0.395	31.7	91.2
3 (3.2 μm)	0.296	23.7	59.5
4 (1.8 μm)	0.205	16.5	35.8
5 (1.0 μm)	0.120	9.7	19.3
6 (0.56 μm)	0.069	5.6	9.6
7 (0.32 μm)	0.025	2.0	4.0
8 (0.18 μm)	0.012	1.0	2.0
9 (0.10 μm)	0.007	0.6	1.0
10 (0.056 μm)	0.003	0.2	0.4
11 (0.032 μm)	0.002	0.2	0.2
12 (0.018 μm)	0.000	0.0	0.0
13 (0.010 μm)	0.000	0.0	0.0
final (～0.010 μm)	0.000	0.0	0.0
Total	1.243	100.0	—

APPENDIX 2

ENVIRONMENTAL CONDITIONS OF INHALATION CHAMBER IN THE 13-WEEK INHALATION STUDY OF MWCNT

ENVIRONMENTAL CONDITIONS OF INHALATION CHAMBER
IN THE 13-WEEK INHALATION STUDY OF MWCNT

Exposure period

Group Name	Temperature (℃) Mean ± S.D.	Humidity (%) Mean ± S.D.	Ventilation Rate (L/min) Mean ± S.D.	Air Change (time/h) Mean
Control	21.6 ± 0.3	53.3 ± 1.4	249.4 ± 2.0	12.1
0.2 mg/m^3	21.8 ± 0.4	52.7 ± 1.6	249.5 ± 2.5	12.1
1 mg/m^3	21.7 ± 0.4	52.9 ± 1.7	249.2 ± 2.5	12.1
5 mg/m^3	21.7 ± 0.4	52.8 ± 1.9	251.8 ± 2.3	12.2

APPENDIX 3

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 13-WEEK INHALATION STUDY OF MWCNT

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 13-WEEK INHALATION STUDY OF MWCNT

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

1) Automatic blood cell analyzer (ADVIA120：Siemens Healthcare Diagnostics Inc.)
2) Automatic analyzer (Hitachi 7080：Hitachi,Ltd.)

APPENDIX 4

METHODS, UNITS AND DECIMAL PLACE FOR CYTOLOGY AND BIOCHEMISTRY OF BALF IN THE 13-WEEK INHALATION STUDY OF MWCNT

METHODS, UNITS AND DECIMAL PLACE FOR CYTOLOGY AND BIOCHEMISTRY OF BALF IN THE 13-WEEK INHALATION STUDY OF MWCNT

Item	Method	Unit	Decimal place
Cytology			
Total cell count	Light scattering method [1)]	$\times 10^3/\mu L$	2
Differential	Visual observation method (May-Grunwald-Giemsa stain)	%	1
Biochemistry			
Total protein(TP)	Pyrogallol red method [2)]	μg/mL	0
Albumin (Alb)	Immuno-nephelometry [2)]	μg/ mL	0
Lactate dehydrogenase (LDH)	JSCC method [2)]	U/L	0
Alkaline phosphatase (ALP)	JSCC method [2)]	U/L	0

1) Automatic blood cell analyzer (ADVIA120：Siemens Healthcare Diagnostics Inc.)
2) Automatic analyzer (Hitachi 7080：Hitachi,Ltd.)