

Supporting Information for

Experimental approaches to data generation for REACH compliance of multi-walled carbon nanotubes: Human health *in vitro/in chemico*

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CONTENTS

1	Test material characterisation	2
2	<i>In vitro</i> serious eye damage/irritation	4
2.1	<i>In vitro</i> eye hazard identification according to OECD TG 492B (adopted 30 June 2022) and in compliance with GLP	4
3	<i>In vitro</i> gene mutation study in mammalian cells.....	9
3.1	K-Nanos 100 MWCNTs: Gene mutation assay in Chinese hamster V79 cells <i>in vitro</i> (V79/HPRT) according to OECD TG 476 (adopted 29 July 2016) and in compliance with GLP	9
3.2	Jenotube 8 MWCNTs: Gene mutation assay in Chinese hamster V79 cells <i>in vitro</i> (V79/HPRT) according to OECD TG 476 (adopted 29 July 2016) and in compliance with GLP	12
	Abbreviation	14

TABLES

Table S 1:	K-Nanos MWCNTs summary of substance characteristics	2
Table S 2:	Jenotube MWCNTs summary of substance characteristics	3
Table S 3:	Result of the test item K-Nanos 300 MWCNTs [30 min].....	4
Table S 4:	Result of the test item K-Nanos 300 MWCNTs [120 min].....	5
Table S 5:	Result of the NSMTT control [30 min].....	6
Table S 6:	Result of the NSMTT control [120 min].....	7
Table S 7:	Validity criteria	7
Table S 8:	K-Nanos 100 MWCNTs summary of results / 4-hour treatment / – S9 mix	9
Table S 9:	K-Nanos 100 MWCNTs summary of results / 4-hour treatment / + S9 mix	10
Table S 10:	K-Nanos 100 MWCNTs summary of results / 24-hour treatment / – S9 mix	11
Table S 11:	Jenotube 8 MWCNTs summary of results / 4-hour treatment /– S9 mix	12
Table S 12:	Jenotube 8 MWCNTs summary of results / 4-hour treatment / + S9 mix	13
Table S 13:	Jenotube 8 MWCNTs summary of results / 24-hour treatment / – S9 mix	14

FIGURES

Figure S 1:	The images show 80 mg of K-Nanos 300 MWCNTs weighed into a 15 mL Falcon tube and an empty tissue insert.....	9
Figure S 2:	Topical application of K-Nanos 300 MWCNTs onto the RHCE tissue surface to ensure complete coverage (left: 30 min; right: 120 min).....	9

1 TEST MATERIAL CHARACTERISATION

Table S 1: K-Nanos MWCNTs summary of substance characteristics

Parameter	K- Nanos 100	K- Nanos 210	K- Nanos 300
Purity [% (w/w)]	94.00~97.00	91.95~98.00	95.00~98.60
Impurity, elements	Al, Co, Fe, Mg, Mo, V		
Single tube length [μm]	Mean: 46.22 Min.: 16.61 Max.: 89.89 D10: 25.44 D50: 46.59 D90: 78.43	Mean: 51.59 Min.: 25.22 Max.: 137.44 D10: 27.34 D50: 45.91 D90: 96.48	Mean: 62.76 Min.: 30.31 Max.: 134.81 D10: 37.50 D50: 55.42 D90: 119.68
Single tube diameter [nm]	Mean: 12.2 Min.: 7.68 Max.: 17.46 D10: 8.93 D50: 11.87 D90: 15.79	Mean: 12.13 Min.: 7.28 Max.: 19.68 D10: 8.05 D50: 12.08 D90: 16.30	Mean: 14.58 Min.: 7.36 Max.: 24.12 D10: 9.63 D50: 14.69 D90: 19.69
Aspect ratio [-] (<i>single tube length / single tube diameter</i>)	Mean: 3789 Min.: 2162 Max.: 5149	Mean: 4253 Min.: 3465 Max.: 6983	Mean: 4304 Min.: 4118 Max.: 5589
Bundle length [μm]	Mean: 41.997 Min.: 15.972 Max.: 70.859 D10: 24.47 D50: 43.30 D90: 61.82	Mean: 48.068 Min.: 26.239 Max.: 113.09 D10: 28.442 D50: 43.401 D90: 79.389	Mean: 54.852 Min.: 28.332 Max.: 104.916 D10: 35.055 D50: 49.980 D90: 93.144
Bundle diameter [μm]	Mean: 3.211 Min.: 0.445 Max.: 12.802 D10: 0.99 D50: 2.45 D90: 6.39	Mean: 2.968 Min.: 0.886 Max.: 15.441 D10: 1.168 D50: 2.207 D90: 5.327	Mean: 2.460 Min.: 0.70 Max.: 8.297 D10: 1.156 D50: 1.774 D90: 5.238
Number of walls	6–24	5–17	5–25
Crystallinity [%]	97.00~98.40	97.80~98.60	97.00~98.20
Surface area [m^2/g]	Mean: 200.8 Min.: 152 Max.: 287	Mean: 247.7 Min.: 202 Max.: 298	Mean: 228.3 Min.: 152 Max.: 348
Zeta potential [mV] (<i>in Ethanol</i>)	-0.459	-0.122	-0.176
Surface chemistry	no	no	no
Relative density [unitless]	1.761 \pm 0.030	-	-
Bulk density [g/cm^3]	0.009–0.030	0.007–0.030	0.005–0.030

Note: D10, D50, D90: 10th, 50th, and 90th percentile of the particle size distribution.

Table S 2: Jenotube MWCNTs summary of substance characteristics

Parameter	Jenotube 6	Jenotube 8	Jenotube 10A	Jenotube 10B
Purity [% (w/w)]	98~99.2	98.5~99.7	97.8~99.2	97.3~98.9
Impurity, elements	Al, Co, Fe, Mg			
Single tube length [μm]	Mean: 77.62 Min.: 11.99 Max.: 238.86 D10: 30.10 D50: 68.34 D90: 151.17	Mean: 136.37 Min.: 2.00 Max.: 579.94 D10: 56.73 D50: 86.36 D90: 343.44	Mean: 35.87 Min.: 9.47 Max.: 107.21 D10: 15.01 D50: 28.60 D90: 66.40	Mean: 50.75 Min.: 10.92 Max.: 122.41 D10: 25.48 D50: 44.20 D90: 92.98
Single tube diameter [nm]	Mean: 7.4 ± 2.5 Min.: 4.4 Max.: 12.8 D10: 4.72 D50: 6.90 D90: 11.5	Mean: 8.04 ± 2.1 Min.: 5.1 Max.: 11.6 D10: 5.24 D50: 7.8 D90: 11.6	Mean: 11.0 ± 2.5 Min.: 7.9 Max.: 14.2 D10: 7.9 D50: 11.6 D90: 14.1	Mean: 12.2 ± 3.3 Min.: 8.3 Max.: 17.9 D10: 8.8 D50: 11.9 D90: 15.9
Aspect ratio [-] (<i>single tube length / single tube diameter</i>)	Mean: 10490 Min.: 2726 Max.: 18661	Mean: 16961 Min.: 391 Max.: 49995	Mean: 3261 Min.: 1199 Max.: 7550	Mean: 4160 Min.: 1316 Max.: 6838
Bundle length [μm]	Mean: 68.6 ± 48.6 Min.: 10.6 Max.: 211.1 D10: 26.6 D50: 60.4 D90: 133.6	Mean: 129.8 ± 123.0 Min.: 1.9 Max.: 552.0 D10: 54.0 D50: 82.2 D90: 326.9	Mean: 35.6 ± 21.0 Min.: 9.4 Max.: 106.4 D10: 14.85 D50: 28.39 D90: 65.87	Mean: 48.8 ± 22.2 Min.: 10.5 Max.: 117.7 D10: 24.5 D50: 42.5 D90: 89.4
Bundle diameter [μm]	Mean: 2.9 ± 1.7 Min.: 0.6 Max.: 8.8 D10: 1.58 D50: 2.48 D90: 5.31	Mean: 3.0 ± 2.3 Min.: 0.33 Max.: 7.93 D10: 0.39 D50: 2.53 D90: 5.59	Mean: 6.3 ± 4.4 Min.: 1.1 Max.: 15.24 D10: 1.4 D50: 4.5 D90: 14.5	Mean: 5.7 ± 3.2 Min.: 1.1 Max.: 15.4 D10: 2.6 D50: 5.2 D90: 10.1
Number of walls	3–9	5–8	5–12	7–18
Crystallinity [%]	95.80	96.99	96.99	97.80
Surface area [m^2/g]	Mean: 644 Min.: 576 Max.: 742	Mean: 570 Min.: 541 Max.: 618	Mean: 257 Min.: 223 Max.: 278	Mean: 217 Min.: 181 Max.: 249
Zeta potential [mV] (<i>with PVP in aqueous solution</i>)	-58.2	-20.9	-53.5	-28.4
Surface chemistry	no	no	no	no
Relative density [unitless]	-	2.294 (2.174–2.414)	-	-
Bulk density [g/cm^3]	ca. 0.025	ca. 0.008	ca. 0.013	ca. 0.065

Note: D10, D50, D90: 10th, 50th, and 90th percentile of the particle size distribution.

2 IN VITRO SERIOUS EYE DAMAGE/IRRITATION

2.1 In vitro eye hazard identification according to OECD TG 492B (adopted 30 June 2022) and in compliance with GLP

- Reconstructed Human Cornea-like Epithelium (RHCE) Test Method for Eye Hazard Identification (Time-to-Toxicity Test (TTT)) with K-Nanos 300 MWCNTs

Table S 3: Result of the test item K-Nanos 300 MWCNTs [30 min]

Name	Negative Control		Positive Control		Test Item	
	1	2	1	2	1	2
Replicate Tissue						
Absolute OD ₅₇₀	1.8516	1.9679	1.3097	1.4197	1.9081	2.0253
	1.8886	1.9807	1.3448	1.4599	1.9969	2.0213
Mean Absolute OD ₅₇₀	1.922****		1.384		1.988	
OD ₅₇₀ (Blank Corrected)	1.807	1.923	1.265	1.375	1.863	1.980
	1.844	1.936	1.300	1.415	1.952	1.976
Mean OD ₅₇₀ of Duplicates (Blank Corrected)	1.825	1.929	1.282	1.395	1.908	1.978
SD OD ₅₇₀ of Duplicates	0.026	0.009	0.025	0.028	0.063	0.003
Total Mean OD ₅₇₀ of 2 Replicate Tissues (Blank Corrected)	1.877*		1.339		1.943	
TOD _{TT}	-		-		1.940	
SD of Mean OD ₅₇₀ of 2 Replicate Tissues (blank corrected)	0.074		0.080		0.050	
Relative Tissue Viability [%]	97.2	102.8	68.3	74.3	101.6	105.4
Mean Relative Tissue Viability [%]	100.0		71.3**		103.5	
Relative Tissue Viability Difference [%]***	5.6		6.0		3.8	
Mean Relative Tissue Viability [%] - NSMTT Corrected	-		-		103.4	

* Blank-corrected mean OD₅₇₀ of the negative control corresponds to 100 % absolute tissue viability.

** Mean relative tissue viability of the three positive control tissues after 30 min is > 40 %.

*** Relative tissue viability difference of replicate tissues is < 20 %.

**** The mean absolute OD₅₇₀ of the negative control is > 1.0 and ≤ 2.5.

Table S 4: Result of the test item K-Nanos 300 MWCNTs [120 min]

Name	Negative Control		Positive Control		Test Item	
	1	2	1	2	1	2
Replicate Tissue						
Absolute OD ₅₇₀	1.826	1.764	0.945	1.149	1.980	1.951
	1.850	1.994	1.003	1.061	2.073	1.929
Mean Absolute OD ₅₇₀	1.858****		1.040		1.983	
OD ₅₇₀ (Blank Corrected)	1.781	1.719	0.900	1.104	1.935	1.906
	1.805	1.949	0.959	1.016	2.028	1.884
Mean OD ₅₇₀ of Duplicates (Blank Corrected)	1.793	1.834	0.929	1.060	1.982	1.895
SD OD ₅₇₀ of Duplicates	0.017	0.162	0.041	0.062	0.066	0.016
Total Mean OD ₅₇₀ of 2 Replicate Tissues (Blank Corrected)	1.813*		0.995		1.939	
TOD _{TT}	-		-		1.939	
SD of Mean OD ₅₇₀ of 2 Replicate Tissues (blank corrected)	0.029		0.092		0.061	
Relative Tissue Viability [%]	98.9	101.1	51.2	58.5	109.3	104.5
Mean Relative Tissue Viability [%]	100.0		54.9**		106.9	
Relative Tissue Viability Difference [%]***	2.3		7.2		4.8	
Mean Relative Tissue Viability [%] - NSMTT Corrected	-		-		106.9	

- * Blank-corrected mean OD₅₇₀ of the negative control corresponds to 100 % absolute tissue viability.
 ** Mean relative tissue viability of the three positive control tissues after 120 min is > 20 % and ≤ 70 %.
 *** Relative tissue viability difference of replicate tissues is < 20 %.
 **** The mean absolute OD₅₇₀ of the negative control is > 1.0 and ≤ 2.5.

Table S 5: Result of the NSMTT control [30 min]

NSMTT	KU		KT		Negative Control	
	1	2	1	2	1	2
Replicate Tissue						
Absolute OD ₅₇₀	0.109	0.104	0.114	0.106	1.945	1.954
	0.099	0.089	0.099	0.093	1.957	1.984
Mean Absolute OD ₅₇₀	0.100		0.103		1.960****	
OD ₅₇₀ (Blank Corrected)	0.029	0.024	0.033	0.026	1.865	1.873
	0.018	0.008	0.018	0.013	1.877	1.904
Mean OD ₅₇₀ of 2 Aliquots (Blank Corrected)	0.023	0.016	0.026	0.019	1.871	1.888
Total Mean OD ₅₇₀ of 2 Replicate Tissues (Blank Corrected)	0.020		0.022		1.879*	
SD OD ₅₇₀ of 2 Replicate Tissues	0.005		0.005		0.013	
NSMTT [%]	0.1				-	
Relative Tissue Viability [%]	1.2	0.8	1.4	1.0	99.5	100.5
Relative Tissue Viability Difference [%]**	0.4		0.4		0.9	
Mean Relative Tissue Viability [%]					100.0	
SD Tissue Viability [%]					0.7	

* Blank-corrected mean OD₅₇₀ of the negative control corresponds to 100 % absolute tissue viability.

** Relative tissue viability difference of replicate tissues is < 20 %.

**** The mean absolute OD₅₇₀ of the negative control is > 1.0 and ≤ 2.5.

Table S 6: Result of the NSMTT control [120 min]

NSMTT Replicate Tissue	KU		KT		Negative Control	
	1	2	1	2	1	2
Absolute OD ₅₇₀	0.104	0.096	0.097	0.095	1.943	1.827
	0.103	0.097	0.104	0.102	1.982	1.865
Mean Absolute OD ₅₇₀	0.100		0.099		1.904****	
OD ₅₇₀ (Blank Corrected)	0.020	0.012	0.013	0.011	1.858	1.743
	0.018	0.013	0.020	0.018	1.898	1.781
Mean OD ₅₇₀ of 2 Aliquots (Blank Corrected)	0.019	0.013	0.016	0.014	1.878	1.762
Total Mean OD ₅₇₀ of 2 Replicate Tissues (Blank Corrected)	0.016		0.015		1.820*	
SD OD ₅₇₀ of 2 Replicate Tissues	0.005		0.001		0.082	
NSMTT [%]	0.0				-	
Relative Tissue Viability [%]	1.0	0.7	0.9	0.8	103.2	96.8
Relative Tissue Viability Difference [%]**	0.3		0.1		6.4	
Mean Relative Tissue Viability [%]					100.0	
SD Tissue Viability [%]					4.5	

* Blank-corrected mean OD₅₇₀ of the negative control corresponds to 100 % absolute tissue viability.

** Relative tissue viability difference of replicate tissues is < 20 %.

**** The mean absolute OD₅₇₀ of the negative control is > 1.0 and ≤ 2.5.

Table S 7: Validity criteria

	Value	Cut off	pass/fail
Mean Absolute OD _{570 nm} NC (30 min Experiment)	1.922 [main exp.] 1.960 [NSMTT-control]	1.0 < NC ≤ 2.5	pass
Mean Absolute OD _{570 nm} NC (120 min Experiment)	1.858 [main exp.] 1.904 [NSMTT control]	1.0 < NC ≤ 2.5	pass
Mean Relative Viability PC [%]; (30 min Experiment)	71.3	> 40 %	pass
Mean Relative Viability PC [%]; (120 min Experiment)	54.9	> 20 %; ≤ 70 %	pass
Max. Difference of Tissue Viability [%]	7.2	< 20 %	pass



Figure S 1: The images show 80 mg of K-Nanos 300 MWCNTs weighed into a 15 mL Falcon tube and an empty tissue insert.

The images clearly demonstrate that the required 80 mg of test item significantly exceeds the volume of the empty tissue insert.

				%	%	%	10 ⁶ cells			regression	
Column	1	2	3	4	5	6	7	8	9	10	
Main Experiment / 4 h treatment				mean values of culture I and II							
Solvent control **			-	100.0	100.0	100.0	4.8	3.3 - 21.3			
Positive control (EMS)	300.0		-	95.5	99.7	95.2	175.3	101.3 - 377.1	0.000 ^S		
Test item	0.4	-	-	105.7	81.2	85.6		#			
Test item	1.0	-	-	107.1	89.1	95.5	11.1	3.3 - 21.3	0.067		
Test item	2.6	P	-	104.2	73.2	76.2		#			
Test item	6.6	P	-	105.1	79.5	83.5	4.7	3.3 - 21.3	n.c.		
Test item	16.4	P	-	106.4	89.0	94.7		#		0.790	
Test item	41.0	P	-	107.7	83.1	90.5	7.0	3.3 - 21.3	0.206		
Test item	102.4	P	-	103.2	80.8	82.5	12.5	3.3 - 21.3	0.007 ^S		
Test item	256.0	P	-	107.3	84.3	90.6	4.6	3.3 - 21.3	n.c.		
Negative Control***			-	94.7	101.0	95.6	6.3	3.3 - 21.3	0.320		

* statistical analysis based on the mean values of culture I and II

** 0.05 % (w/v) BSA–water solution

*** 10 % water in culture medium

n.c. not calculated (mean MF below the upper limit of the 95 % control limit)

P precipitation visible at the end of treatment

S significant trend (p < 0.05)

MF Mutant frequency

cultures not continued as a minimum of only four analysable concentrations are required

Table S 9: K-Nanos 100 MWCNTs summary of results / 4-hour treatment / + S9 mix

	conc. µg/mL	P	S9 mix	relative	relative	rel.	(MF)	95 %	statistical	
				cloning efficiency I %	cell density %	adjusted cloning efficiency I %	mutant colonies/ 10 ⁶ cells	control limit	t-test	linear regression
Column	1	2	3	4	5	6	7	8	9	10
Main Experiment / 4 h treatment				mean values of culture I and II						
Solvent control **			+	100.0	100.0	100.0	4.1	3.4 - 22.5		
Positive control (DMBA)	2.3		+	94.3	142.0	139.0	69.6	26.7 – 201.0	0.000 ^S	
Test item	0.4	-	+	112.2	147.9	168.5	8.8	3.4 - 22.5	0.040 ^S	
Test item	1.0	P	+	105.4	148.8	159.7		#		
Test item	2.6	P	+	108.7	137.2	152.5		#		
Test item	6.6	P	+	103.1	133.4	138.8	7.5	3.4 - 22.5	0.019 ^S	
Test item	16.4	P	+	106.5	125.0	137.1		#		0.759
Test item	41.0	P	+	108.4	114.2	124.6	8.1	3.4 - 22.5	0.006 ^S	
Test item	102.4	P	+	105.2	93.1	100.7	10.5	3.4 - 22.5	0.036 ^S	
Test item	256.0	P	+	107.8	115.6	127.5	7.2	3.4 - 22.5	0.084	
Negative control***			+	105.3	158.4	170.1	5.4	3.4 - 22.5	0.307	

* statistical analysis based on the mean values of culture I and II

** 0.05 % (w/v) BSA–water solution

*** 10 % water in culture medium

n.c. not calculated (mean MF below the upper limit of the 95 % control limit)

P precipitation visible at the end of treatment

S significant trend (p < 0.05)

MF Mutant frequency

cultures not continued as a minimum of only four analysable concentrations are required

Table S 10: K-Nanos 100 MWCNTs summary of results / 24-hour treatment / – S9 mix

	conc. µg/mL	P	S9 mix	relative	relative	rel.	(MF)	95 %	statistical	
				cloning efficiency I %	cell density %	adjusted cloning efficiency I %	mutant colonies/ 10 ⁶ cells	control limit	t-test	linear regres sion
Column	1	2	3	4	5	6	7	8	9	10
Main Experiment / 24 h treatment				mean values of culture I and II						
Solvent control **			-	100.0	100.0	100.0	9.7	2.7–26.5		
Positive control (EMS)	214.0		-	90.6	89.0	81.0	726.3	106.3 – 598.7	0.000 ^S	
Test item	0.4	-	-	87.0	111.4	95.4	11.1	2.7–26.5	0.546	
Test item	1.0	P	-	86.9	99.3	83.5		#		
Test item	2.6	P	-	87.2	101.6	88.0		#		
Test item	6.6	P	-	86.6	109.4	93.9	14.3	2.7–26.5	0.336	
Test item	16.4	P	-	86.3	105.1	89.0		#		0.458
Test item	41.0	P	-	85.0	99.9	82.5	11.5	2.7–26.5	0.271	
Test item	102.4	P	-	90.8	89.0	80.1	9.4	2.7–26.5	n.c.	
Test item	256.0	P	-	88.8	90.0	79.0	14.1	2.7–26.5	0.090	
Negative control***			-	91.5	106.8	96.3	8.1	2.7–26.5	n.c.	

- * statistical analysis based on the mean values of culture I and II
- ** 0.05 % (w/v) BSA–water solution
- *** 10 % water in culture medium
- n.c. not calculated (mean MF below the upper limit of the 95 % control limit)
- P precipitation visible at the end of treatment
- S significant trend (p < 0.05)
- MF Mutant frequency
- # cultures not continued as a minimum of only four analysable concentrations are required

3.2 Jenotube 8 MWCNTs: Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) according to OECD TG 476 (adopted 29 July 2016) and in compliance with GLP

Table S 11: Jenotube 8 MWCNTs summary of results / 4-hour treatment /– S9 mix

Column	conc. µg/mL	P	S9 mix	relative	relative	rel. adjusted	MF	95 %	Statistical analysis		
				cloning efficiency I %	cell density %	cloning efficiency I %	mutant colonies/ 10 ⁶ cells	confidence interval	t-test	linear regressi on	
1	2	3		4	5	6	7	8	9	10	
4 h treatment				mean values of culture I and II							
Solvent control*			-	100.0	100.0	100.0	13.4	2.9 - 22.4			
Positive control (EMS)	300.0		-	90.1	96.3	87.4	172.4	--	0.000 ^S		
Test item	0.4	-	-	97.0	91.9	89.5		#			
Test item	1.0	-	-	93.8	99.7	93.2	7.1	2.9 - 22.4	n.c.		
Test item	2.6	P	-	101.4	113.0	114.5		#			
Test item	6.6	P	-	94.4	100.2	95.0		#			
Test item	16.4	P	-	96.3	97.6	93.7	7.3	2.9 - 22.4	n.c.		
Test item	41.0	P	-	95.6	83.7	80.7	6.6	2.9 - 22.4	n.c.	0.635	
Test item	102.4	P	-	88.1	80.3	69.4	9.4	2.9 - 22.4	n.c.		
Test item	256.0	P	-	81.8	92.7	75.7	10.7	2.9 - 22.4	n.c.		
Negative control**			-	103.4	110.9	115.7	12.0	2.9 - 22.4	n.c.		

* 0.05 % (w/v) BSA–water solution

** 10 % water in culture medium

n.c. not calculated (mean MF below the upper limit of the 95 % control limit)

P precipitation at the end of treatment

S significant trend (p < 0.05)

MF Mutant frequency

Table S 12: Jenotube 8 MWCNTs summary of results / 4-hour treatment / + S9 mix

Column	conc. µg/mL	P	S9 mix	relative cloning efficiency I %	relative cell density %	rel. adjusted cloning efficiency I %	MF mutant colonies/ 10 ⁶ cells	95 % confidence interval	Statistical analysis	
									t-test	linear regression
1	2	3	4	5	6	7	8	9	10	
4 h treatment			mean values of culture I and II							
Solvent control			+	100.0	100.0	100.0	10.8	2.9 - 23.7		
Positive control (DMBA)	2.3		+	90.0	94.2	85.1	73.1	--	0.000 ^S	
Test item	0.4	-	+	98.9	99.2	98.1		#		
Test item	1.0	-	+	89.1	87.2	78.0		#		
Test item	2.6	-	+	87.4	92.1	80.3	8.0	2.9 - 23.7	n.c.	
Test item	6.6	P	+	92.0	75.8	70.3		#		
Test item	16.4	P	+	84.6	93.1	77.9	8.8	2.9 - 23.7	n.c.	0.833
Test item	41.0	P	+	90.1	72.8	65.5	16.1	2.9 - 23.7	n.c.	
Test item	102.4	P	+	92.4	62.9	58.3	7.8	2.9 - 23.7	n.c.	
Test item	256.0	P	+	77.9	71.4	55.5	11.5	2.9 - 23.7	n.c.	
Negative control**			+	103.4	94.2	97.4	13.4	2.9 - 23.7	n.c.	

* 0.05 % (w/v) BSA–water solution

** 10% water in culture medium

n.c. not calculated (mean MF below the upper limit of the 95 % control limit)

P precipitation at the end of treatment

S significant trend (p < 0.05)

MF Mutant frequency

Table S 13: Jenotube 8 MWCNTs summary of results / 24-hour treatment / – S9 mix

	conc. µg/mL	P	S9 mix	relative cloning efficiency I %	relative cell density %	rel. adjusted cloning efficiency I %	MF mutant colonies/ 10 ⁶ cells	95 % confidence interval	Statistical analysis		
									t-test	linear regression	
Column	1	2	3	4	5	6	7	8	9	10	
24 h treatment				mean values of culture I and II							
Solvent control*			-	100.0	100.0	100.0	9.1	2.7 – 26.5			
Positive control (EMS)	214.0		-	90.4	72.2	65.1	233.1	--	0.000 ^S		
Test item	0.4	P	-	99.0	91.1	90.2		#			
Test item	1.0	P	-	106.9	86.4	92.4		#			
Test item	2.6	P	-	104.5	81.4	85.1		#			
Test item	6.6	P	-	102.7	61.1	63.0	8.2	2.7 – 26.5	n.c.		
Test item	16.4	P	-	105.7	75.0	78.9	8.7	2.7 – 26.5	n.c.		
Test item	41.0	P	-	101.6	61.7	62.6	9.6	2.7 – 26.5	n.c.		
Test item	102.4	P	-	101.8	49.4	49.7	6.0	2.7 – 26.5	n.c.	0.573	
Test item	256.0	P	-	104.4	39.1	41.0	8.3	2.7 – 26.5	n.c.		
Negative control**			-	106.6	88.7	94.3	9.7	2.7 – 26.5	n.c.		

* 0.05 % (w/v) BSA–water solution

** 10 % water in culture medium

n.c. not calculated (mean MF below the upper limit of the 95 % control limit)

P precipitation at the end of treatment

S significant trend (p < 0.05)

MF Mutant frequency

culture not continued as a minimum of only four analysable concentrations are required

ABBREVIATION

BSA – Bovine Serum Albumin

GLP – Good Laboratory Practice

MF – Mutant Frequency

MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide

MWCNTs – Multi-Walled Carbon Nanotubes

NSMTT – Non-Specific MTT

OD – Optical Density

PVP – Polyvinylpyrrolidone