

# **Comparative cytotoxicity of respirable surface-treated/untreated calcium carbonate rock dust particles *in vitro***

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## Supplementary Material

### Estimation of human equivalent exposure for type II alveolar cells (A549)

Given that:

MSHA allowable exposure to coal mine dusts with silica <5% (**A**) = 1.5 mg/m<sup>3</sup> (respirable fraction)

Daily breathing volume (**B**) = 20 L/min = 20L/min \* 0.001 m<sup>3</sup>/L \* 60 min/hr \* 8hr/day = 9.6 m<sup>3</sup>/day

Average deposition efficiency of particles of size ~1-2 mm (**C**) = 15% = 0.15

Total number of type II epithelial cells in the alveolar space of the human lung (**D**) = 32,900 ±13,600 (x 10<sup>6</sup>) cells (Stone et al., 1992)

Dosage used *in vitro* :

If 15625 of the A549 cells (per well) were exposed to 2.5 µg (0.025 mg/ml in 100 µl) of rock dust particles in 96 well flask.

Deposited Concentration per cell (**E**) = 2.5µg / 15625 cells = 0.00016 µg per cell

Equivalent exposure duration in humans = (E x D) / (A x B x C) =

= (0.00016 µg/cell x (32,900 x 10<sup>6</sup> cells)) / (1.5 mg/m<sup>3</sup> x 9.6 m<sup>3</sup>/day x 0.15) =

= (5,264 mg) / ( 2.16 mg/day) = ~2,437 working days = ~9 years

### Estimation of human equivalent exposure for alveolar macrophages (THP-1)

Given that:

MSHA allowable exposure to coal mine dusts with silica <5% (**A**) = 1.5 mg/m<sup>3</sup> (respirable fraction)

Daily breathing volume (**B**) = 20 L/min = 20L/min \* 0.001 m<sup>3</sup>/L \* 60 min/hr \* 8hr/day = 9.6 m<sup>3</sup>/day

Average deposition efficiency of particles of size ~1-2 mm (**C**) = 15% = 0.15

Total number of macrophages in the alveolar space of the human lung (**D**) = 5,990 ±1,900 (x 10<sup>6</sup>) cells (Stone et al., 1992)

Dosage used *in vitro* :

If 15625 of the THP-1 cells (per well) were exposed to 2.5 µg (0.01 mg/ml in 100 µl) of rock dust particles in 96 well flask.

Deposited Concentration per cell (**E**) = 2.5µg / 15625 cells = 0.00016 µg per cell

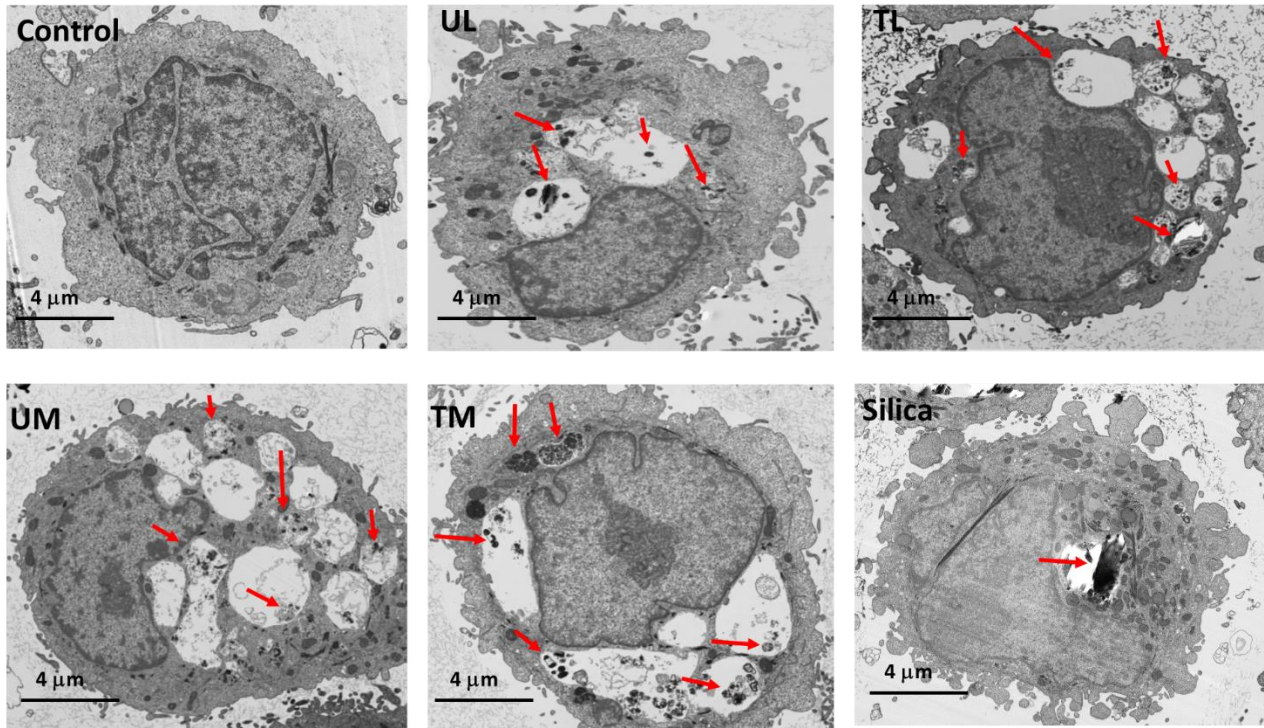
Equivalent exposure duration in humans = (E x D) / (A x B x C) =

= (0.00016 µg/cell x (5,990 x 10<sup>6</sup> cells)) / (1.5 mg/m<sup>3</sup> x 9.6 m<sup>3</sup>/day x 0.15) =

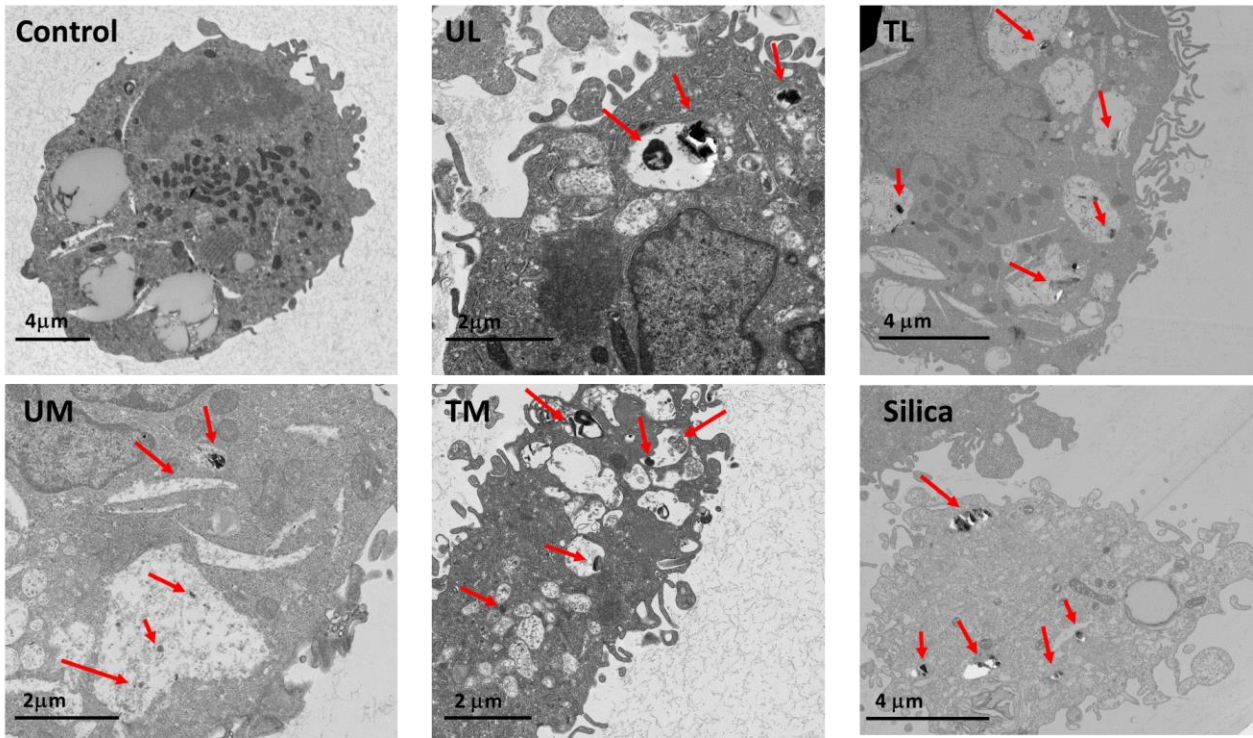
= (958.4 mg) / ( 2.16 mg/day) = ~443.7 working days = ~1.5 years

Sample	Average quartz content, %
UL	0.84±0.22
TL	1.02±0.12
UM	0.08
TM	0.05±0.04

**Supplementary Table 1.** Quartz content in respirable fractions of rock dust



**Supplementary Figure 1.** TEM micrographs of A549 cells exposed to different rock dust or silica for 72 h (0.1 mg/ml). Red arrows indicate particle uptake.



**Supplementary Figure 2.** TEM micrographs of THP-1 cells exposed to different rock dust or silica for 24 h (0.1 mg/ml). Red arrows indicate particle uptake.

	IL-1b	IL-1ra	IL-4	IL-6	IL-7	IL-8	IL-9	IL-10	IL-12(p70)	IL-13	IL-17	Eotaxin	FGF basic	IFN-g	IP-10	MCP-1	MIP-1a	PDGF-bb	MIP-1b	RANTES	TNF-a	
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
UM_0.025	129.6	148.0304	118.1144	132.1914	126.1194	143.4634	106.037	177.9157	111.1969	132.8296	211.781	137.6259	172.4666	123.8985	150.629	92.49643	128.2051	131.4884	155.3861	119.2275	132.062	
UM_0.1	139.2	143.4658	83.68644	139.4458	123.041	159.7156	97.56098	112.1951	136.8726	108.1362	232.6881	154.2684	167.1458	124.6995	160.9208	113.2656	126.2019	112.3459	158.5339	123.1577	148.6599	
UM_0.2	164.8	198.377	158.8983	176.5239	92.35075	251.1314	126.1028	137.4723	147.8764	71.39728	336.1588	186.868	237.1397	234.5534	182.3797	171.2113	173.0769	119.2644	225.0381	140.2677	180.0002	
TM_0.025	144	177.6839	111.2288	151.738	149.1981	153.2443	136.2221	198.3149	140.5405	148.0508	252.2401	168.2365	215.8373	141.181	167.4222	95.70414	134.6154	158.4815	161.3956	133.7456	182.6291	
TM_0.1	144	173.0854	125	153.5516	135.541	169.5565	121.0863	194.7672	147.8764	150.3869	234.9336	163.2637	224.8329	129.4776	164.1927	120.624	144.2308	155.4345	173.1282	136.1342	180.062	
TM_0.2	196.8	244.6999	175.8475	169.2695	181.5299	130.6983	178.1266	145.898	175.9073	148.2698	350.5808	195.2887	320.2318	128.6814	188.9236	125.069	177.8846	200.6022	174.2156	156.0224	200.8765	
UL_0.025	139.2	185.8833	99.04661	149.9244	104.737	139.4814	93.40944	177.9157	134.6718	117.8274	188.1637	153.0307	155.4495	122.3802	141.0766	115.8268	115.3846	85.93825	144.6837	124.784	132.9898	
UL_0.1	139.2	157.0583	120.7627	156.9773	99.36591	142.3421	140.6331	144.2129	140.5405	122.3719	228.7196	157.0531	191.476	134.3658	167.9746	133.9202	132.6923	119.2644	168.8358	121.5653	162.5775	
UL_0.2	116.8	148.3686	143.0085	123.3249	116.2253	96.2675	160.8718	81.89209	128.8031	138.0859	164.0044	123.923	231.6017	137.7422	155.8981	150.1015	129.8077	120.6983	149.5675	109.131	127.8094	
TL_0.025	137.6	172.2739	130.2966	139.2443	110.1828	136.2881	111.0535	150.9534	128.8031	133.8152	231.6234	153.0086	175.7457	119.6077	148.6686	119.2655	122.1154	114.6042	162.7691	117.0591	153.8661	
TL_0.1	121.6	157.1006	118.1144	125.3401	82.50653	113.2566	113.7779	112.6386	128.8031	92.75077	184.5824	129.4484	190.5577	118.9515	138.493	113.6642	104.8077	91.19587	123.0498	111.6043	142.4538	
TL_0.2	147.2	154.3533	139.8305	123.3249	138.3812	115.6331	128.8272	13.5255	120.1544	101.9492	184.292	129.4484	221.7883	146.3279	145.7508	140.3102	110.5769	113.0987	124.7668	107.8096	127.8352	
Sil_0.025	100.8	130.3128	155.7203	104.3829	87.46736	114.0512	112.74	90.64302	122.9344	106.9864	138.2965	113.5795	111.5654	118.4257	129.5185	92.70488	84.61538	110.2667	69.59468	103.8794	115.1754	
Sil_0.1	97.6	139.0025	83.15678	104.3829	102.4804	118.2012	79.52776	70.95344	134.6718	122.5909	140.542	112.165	89.46497	111.3096	138.493	97.99516	90.38462	79.90393	95.29206	106.1833	98.41247	
Sil_0.2	118.4	152.6965	185.3814	113.0479	105.3338	163.8668	161.5205	N/D	140.5405	93.57205	188.0089	114.9498	162.559	148.2175	162.391	133.7591	125	110.2309	122.9353	119.4647	154.9486	

Supplementary Table 2. Cytokine secretion in supernatants of A549 cells exposed to various Rock Dusts or crystalline silica.

	IL-1b	IL-6	IL-12(p70)	G-CSF	GM-CSF	IFN-g	TNF-a	IL-2	IL-4	IL-5	IL-9	IL-10	IL-13	Eotaxin	IP-10	MCP-1(MIP-1a)	MIP-1a	MIP-1b	
Control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
UM_0.025	82.23231	107.9523	151.0412	115.8082	114.6176	127.7058	108.7528	109.7135	111.0519	220.7549	126.7217	115.2272	114.2857	141.8824	104.8782	136.5605	111.3852	133.3271	
UM_0.05	85.74777	104.7714	121.5217	119.6483	107.1903	137.1751	116.1479	131.3767	112.9161	253.3021	120.1585	104.4051	149.8829	121.8142	135.902	125.4448	121.0411	129.9036	
UM_0.1	139.3877	163.3201	122.5973	148.2873	112.3179	138.3479	163.9922	124.8078	131.2916	285.8493	148.0299	117.5858	142.8571	146.3375	111.9995	146.766	178.3634	215.4964	
UM_0.2	113.4173	160.6362	143.4783	166.8243	125.1136	183.0884	180.4434	172.7463	150.7324	502.359	148.7666	136.2123	185.4801	176.8814	132.0421	176.5687	188.805	255.7597	
TM_0.025	250.969	242.3495	135.3066	182.5656	111.3702	153.8704	260.1227	119.7749	140.274	159.8214	163.5563	124.976	118.0812	149.6762	123.5737	115.7241	218.2229	410.1668	
TM_0.05	501.6274	543.1392	159.6533	289.3303	110.735	205.7031	505.8214	167.5241	146.5753	71.42857	170.4728	114.5103	90.0369	183.7333	120.3564	95.47718	227.567	844.0575	
TM_0.1	736.2685	929.1214	162.0803	371.6313	117.102	265.7372	791.486	230.0643	198.0822	96.42857	215.1723	179.5668	143.1734	193.981	146.2099	100.4025	245.9511	1673.066	
TM_0.2	1090.891	1535.439	145.9518	620.4913	130.9121	333.3291	1226.067	290.5145	220.8219	180.3571	189.7384	158.6352	118.0812	209.981	150.7233	99.39286	234.4813	2209.097	
UL_0.025	138.7933	74.59424	96.10197	91.72651	95.54923	92.73098	93.11649	92.08633	97.14937	96.42857	110.6364	87.68962	92.06897	113.2288	96.40959	116.5416	98.58944	95.48739	
UL_0.05	152.7525	95.52832	95.99249	101.5298	97.37401	100.4615	112.2542	118.4283	104.447	139.2857	108.7709	100.0134	94.82759	104.4603	99.58034	118.6333	117.0034	121.8566	
UL_0.1	124.2348	121.4972	107.1119	121.1427	107.4724	133.2742	159.3855	119.3138	113.569	160.7143	135.0985	104.2825	120.6897	129.2739	111.7225	167.5957	160.824	168.2495	
UL_0.2	216.4268	115.6012	103.2881	118.0331	99.09415	137.1439	183.0327	121.5274	120.8666	180.3571	123.5896	99.04685	126.2069	114.9642	99.58034	133.1216	172.3651	183.8234	
TL_0.025	177.0885	77.60417	107.3426	87.42679	100.7134	101.0457	114.5778	88.86986	98.4456	153.8462	107.3802	82.19027	102.9963	101.1579	118.8243	90.70329	106.7462	103.4174	
TL_0.05	126.9914	102.2569	76.52384	97.87434	109.7627	94.59539	129.0472	119.6918	102.0725	253.8462	103.5839	75.35951	79.77528	100.2807	104.9361	109.0554	154.7133	117.7895	
TL_0.1	162.6006	143.9236	125.4074	115.6605	113.3452	123.4461	191.3747	199.6575	124.0933	199.5731	131.2527	101.3551	122.4719	137.193	114.0004	127.0972	194.9041	233.7527	
TL_0.2	117.1385	147.309	116.717	123.1726	112.7792	129.828	180.091	178.5959	127.7202	253.8462	128.1318	99.4469	117.2285	129.1579	119.8564	148.9083	200.007	208.5075	
Sil_0.005	361.6429	442.9818	218.693	213.9218	120.7065	200.2119	773.6704	170.8637	181.8182	165.1288	161.8638	188.8065	151.7241	184.7404	121.1209	195.397	238.3543	568.388	
Sil_0.01	364.1757	288.7273	119.9098	158.7494	101.964	128.8061	241.4724	107.076	140.6566	100.0885	145.9939	117.2286	110.6897	119.5285	98.30875	85.11166	164.6784	306.8592	
Sil_0.025	1571.033	1781.309	140.6902	691.9004	134.8216	295.461	991.4005	216.7534	254.0404	194.8971	188.571	178.8604	167.5862	217.8401	166.3717	116.3896	245.6621	1592.297	
Sil_0.05	4238.152	3011.345	145.6415	1154.992	140.0495	415.2331	1863.046	281.5817	353.2828	130.2874	184.3205	202.9332	146.8966	272.0293	179.882	105.0124	252.4812	1667.132	

Supplementary Table 3. Cytokine secretion in supernatants of THP-1 cells exposed to various Rock Dusts or crystalline silica.

**References:**

Stone KC, Mercer RR, Gehr P, Stockstill B, Crapo JD (1992) Allometric relationships of cell numbers and size in the mammalian lung. *Am J Respir Cell Mol Biol* 6:235-243.