Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information
For

Biodissolution and Cellular Response to MoO_3 Nanoribbons and a New Framework for Early Hazard Screening for 2D Materials

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Table S1. Formulation and reagent information for EPA moderately hard water[1] used in biopersistence assays. The pH range of moderately hard synthetic water is from 7.4 to 7.8.

Component	Molecular Weight (g/mol)	Concentration (mg/L)	mM	Manufacturer	Lot
$NaHCO_3$	84.007	96	1.1	Sigma Aldrich	088K014411
$CaSO_4 \cdot 2H_2O$	172172	60	3.5E-4	Arcos Organics	A0350914
$MgSO_4$	120.336	60	0.50	Sigma Aldrich	099K0089
KCL	74.5513	4	0.054	Fisher Scientific	755344

Table S2. Components of the Roswell Park Memorial Institute Media (RPMI) as reported by ThermoFisher Scientific (LOT 11875-093) used for biopersistence assays. The RMPI solution was supplemented with fetal bovine serum (FBS, Gibco, 1913800) and Penn Strep (Gibco, 1751328) both from Gibco. The pH of this solution was 7.4

Components	Molecular Weight (g/mol)	Concentration (mg/L)	mM
Amino Acids			
Glycine	75	10	0.13
L-Arginine	174	200	1.1
L-Asparagine	132	50	0.38
L-Aspartic Acid	133	20	0.15
L-Cystine 2HCL	313	65	0.21
L-Glutamic Acid	147	20	0.14
L-Glutamine	146	300	2.1
L-Histidine	155	15	0.10
L-Hydroxyproline	131	20	0.15
L-Isoleucine	131	50	0.38
L-Leucine	131	50	0.38
L-Lysine hydrochloride	183	40	0.22
L-Methionine	149	15	0.10
L-Phenylalanine	165	15	0.091
L-Proline	115	20	0.17
L-Serine	105	30	0.29
L-Threonine	119	20	0.17
L-Tryptophan	204	5	0.025
L-Tyrosine disodium salt dihydrate	261	29	0.11
L-Tyrosine	117	20	0.17
Vitamins			
Biotin	244	0.2	8.2E-04
Choline Chloride	140	3	0.021
D-Calcium Pantothenate	477	0.25	5.2E-04
Folic Acid	441	1	0.0023
Niacinamide	122	1	0.0082
Para-Aminobenzoic Acid	137	1	0.0073
Pyridoxine hydrochloride	206	1	0.0049
Riboflavin	376	0.2	5.3E-04
Thiamine hydrochloride	337	1	0.0030
Vitamin B12	1355	0.005	3.7E-06

i-Inositol	180	35	0.19
Inorganic Salts			
Calcium Nitrate (Ca(NO3)2•4H2O)	236	100	0.42
Magnesium Sulfate 9MgSO4 (anhyd.)	120	48.84	0.41
Potassium Chloride (KCL)	75	400	5.3
Sodium Bicarbonate (NaHCO3)	84	2000	24
Sodium Chloride (NaCl)	58	6000	103
Sodium Phosphate Dibasic (Na2HPO4) (anhyd.)	142	800	5.6
Other Components			
D-Glucose (Dextrose)	180	2000	11
Glutathione (reduced)	307	1	0.0033
Phenol Red	376.4	5	0.013

Table S3. Formula of phosphate buffered saline solution (PBS) used in biopersistence assays as listed by Fisher Scientific. This solution was purchased and is listed as a 10X concentrated stock and was diluted to 1X prior to use.

Component	MW	g/L	M
NaCl	58	80.0628	1.4
KCl	75	2.0128851	0.027
Phosphate Buffer	411	48.912451	0.12

Table S4 Individual components used to prepare lung simulant fluid (SLF) using the formula published by Gray et al.[2]

Component	MW	mg/L	mM	Manufacturer	Lot
NaCl	58	6.8	6.8	Fisher Scientific	126479
NH_4Cl	53	5.3	5.3	Fisher Scientific	158618
$NaHCO_3$	84	2.3	2.3	Sigma Aldrich	088K014411
H_3PO_4	98	1.2	1.2	Arcos Organics	AO322150
$NaH_2PO4 \bullet H_2O$	138	1.7	1.7	Fisher Scientific	151189A
Na_2CO_3	106	0.63	0.63	Fisher Scientific	156281
NaAC	82	0.58	0.58	Sigma Aldrich	40K0175
KHP	204	0.2	0.20	Arcos Organics	AO237427
Glycine	75	0.45	0.45	Fisher Scientific	1314121
H_2SO_4	98	0.51	0.51	Fisher Scientific	152112
Na_3-Citrate $\bullet 2H_2O$	258	0.59	0.59	Sigma Aldrich	079K0044
$CaCl_2$	111	0.29	0.29	Fisher Scientific	096799
Citric Acid	192	0.42	0.42	Citric Acid	158634

Table S5. Individual components used to prepare the phagolysosomal simulant fluid (PSF) using the formula published by Stefaniak et al.[3]

Component	MW	mg/L	mM	Manufacturer	Lot
Na_2HPO_4	142	142	1.0	Sigma Aldrich	60790
NaCl	58	6650	114	Fisher	126479
Na_2SO_4	142	71	0.50	Fisher Scientific	09505DE
$CaCl_2 \cdot 2H_2O$	147	29	0.20	Fisher Scientific	096799
Glycine	75	450	6.0	Fisher Scientific	1314121
Potassium hydrogen phthalate (1-(HO2C)–2-(CO2K)–C6H4)	204	4085	20	Arcos Organics	AO237427
Alkylbenzyldimethylammonium chloride (ABCD)	N/A (polymer)	50	-	Alpha Aesar	X03A029

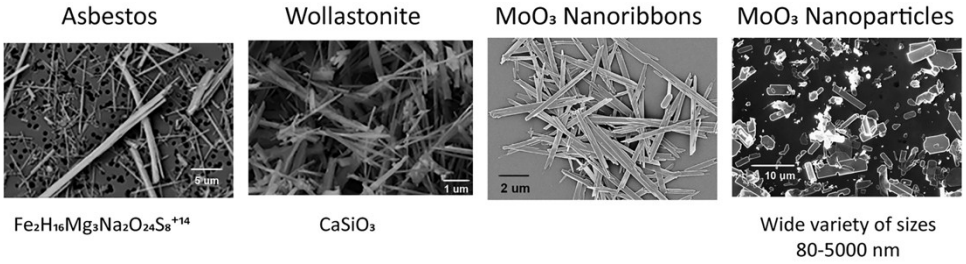

Figure S1. Additional morphology characterization of test and reference samples. (A) Crocidolite asbestos: as reported in Moalli et al. (1987)[4] and Sanchez et al. (2011)[5]. (B) Wollastonite: as reported in Bellman and Muhle (1994)[6] and Macdonald and Kane (1997).[6,7] (C) MoO_3 nanoribbon: SEM image obtained using a Zeiss LEO 1530 at 10 KeV. (D) MoO_3 nanoparticle SEM image obtained using a Zeiss LEO 1530 at 10 KeV.

Table S6. Summary of particulate material characterization.

Material	Size	Shape	Surface Area (m^2/g)	Chemical Composition
MoO_3 nanoribbons	10µm x 200nm x 10nm	Small, lamellar strips	44.82	MoO_3
MoO_3 nanoparticles	80-5000 nm	Rectanglular / irregular	0.03-2	MoO_3
Wollastonite (NYAD 1250 THOR) Belmann and Muhle 1994	0.4µm (1.42) x 1.7 (1.4) µm	Rod-like	3.94	$CaSiO_3$
Crocidolite asbestos (Sanchez et al 2011)	2.8 (2.6) µm x 116 (112) nm	Rod-like	9.1	$(Na_2(Fe^{3+})_2(Fe^{2+})_3Si_8O_{22}(OH)_2)$

Table S7. Concentrations of particlulate and soluble molybdate compounds expressed at equivalent ionic Mo concentrations

MoO_3 concentration (µg/ml)	Mo Molarity (µM)	Na_2MoO_4 concentration (µg/ml)
10	70	14.3
50	350	71.5
100	690	143

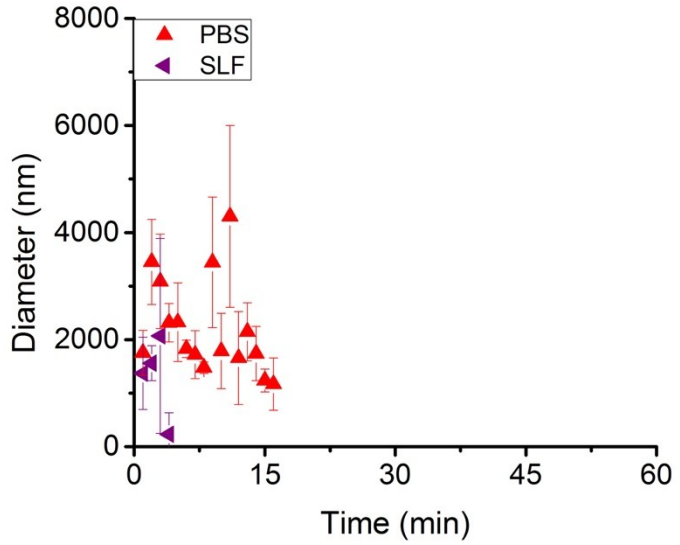

Figure S2. DLS stability test results for MoO_3 suspended in Phosphate Buffered Saline (PBS) and Simulated Lung Fluid (SLF) at a concentration of 500 mg/L. Both systems are buffered at pH 7.4 and contain no proteins (i.e. FBS). Three replicates were taken for each time point. Data shown reflects what was obtained prior to the instrument terminating data collection due to failing instrumental data quality criteria.

MoO_3 Concentration Dependent Behavior

The observed dissolution and stability of MoO_3 in EPA moderately hard water can be explained by comparing total the variable total MoO_3 concentration to the fixed concentration of HCO_3^- in Mod water. Bicarbonate is capable of buffering any H+ produced through the dissolution of MoO3, which is expected based on the initial pH range of 7.4 to 7.8 for Mod water. The molar concentration of HCO_3^- (added as $NaHCO_3$) is fixed at 1.14 mol L^{-1}. Complete dissolution of MoO_3 following the reaction stoichiometry of equation 1 would yield H^+ concentrations of 6.9

mol L^{-1} for DLS experiments and 0.69 mol L^{-1} for filtration experiments. Clearly, DLS experiments would produce enough H+ to protonate all available HCO_3^-, at which point solution pH would decrease and preventing further dissolution by Le Chatelier's principle. The opposite condition exists for filtration experiments, where H+ produced through MoO_3 dissolution does not exceed the buffering capacity of bicarbonate and compete dissolution is achieved prior to any observable pH change. Therefore, reported stability and dissolution are both true, but represent different behavior based on the ratio of H^+ (from MoO_3) to the buffer bicarbonate. Screening level techniques, at best should be able to capture both of these reactions. The present approaches could be expanded to cover a greater range of concentrations (high and low) for each screening to rapidly tease out any concentration dependent persistence behavior

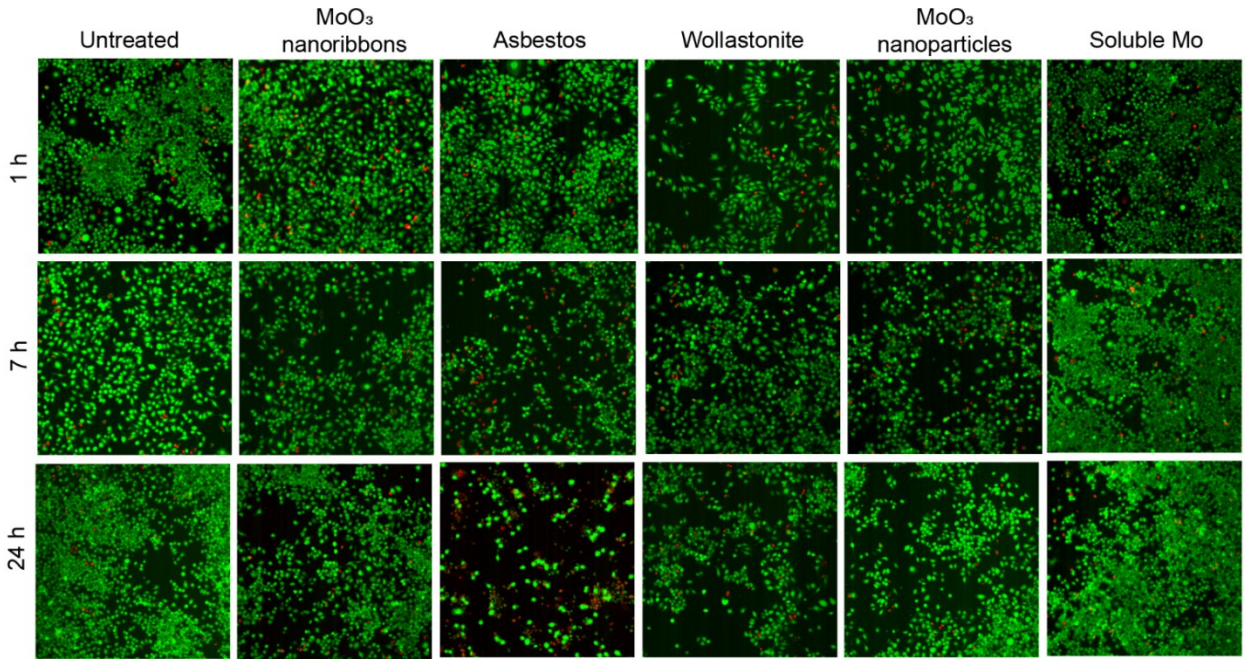

Fig. S3. Representative images of macrophages stained with calcein AM (green) or ethidium homodimer 1 (red) after 1, 7, 24 h exposures. Calcein AM stain (green) = live cells; ethidium homodimer 1 stain (red) = dead cells.

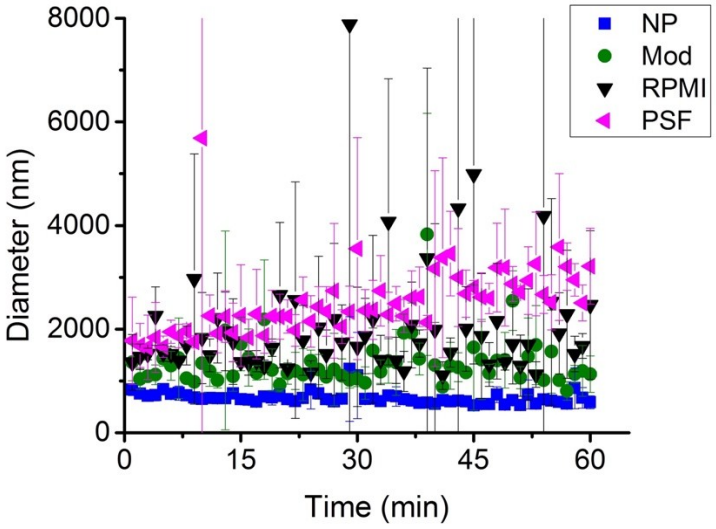

Figure S4. DLS monitoring in four fluid media at nanoribbon starting concentration of 50 mg L^{-1} in NP, Mod, RPMI and PSF.

References

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