

## Supporting Information For

### Biodissolution and Cellular Response to MoO<sub>3</sub> 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<sup>1</sup> 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 <sub>3</sub>                    | 84.007                   | 96                   | 1.1    | Sigma Aldrich     | 088K014411 |
| CaSO <sub>4</sub> • 2H <sub>2</sub> O | 172172                   | 60                   | 3.5E-4 | Arcos Organics    | A0350914   |
| MgSO <sub>4</sub>                     | 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      |
|------------------------------------|--------------------------|----------------------|---------|
| <b>Amino Acids</b>                 |                          |                      |         |
| 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    |
| <b>Vitamins</b>                    |                          |                      |         |
| 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   |
| <b>Inorganic Salts</b>                                                 |       |       |        |
| Calcium Nitrate (Ca(NO <sub>3</sub> ) <sub>2</sub> •4H <sub>2</sub> O) | 236   | 100   | 0.42   |
| Magnesium Sulfate 9MgSO <sub>4</sub> (anhyd.)                          | 120   | 48.84 | 0.41   |
| Potassium Chloride (KCL)                                               | 75    | 400   | 5.3    |
| Sodium Bicarbonate (NaHCO <sub>3</sub> )                               | 84    | 2000  | 24     |
| Sodium Chloride (NaCl)                                                 | 58    | 6000  | 103    |
| Sodium Phosphate Dibasic (Na <sub>2</sub> HPO <sub>4</sub> ) (anhyd.)  | 142   | 800   | 5.6    |
| <b>Other Components</b>                                                |       |       |        |
| 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.

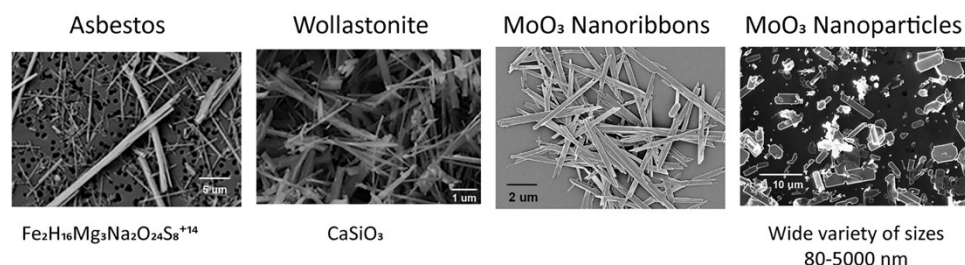
| Component        | MW  | g/L       | M     |
|------------------|-----|-----------|-------|
| NaCl             | 58  | 80.0628   | 1.4   |
| KCl              | 75  | 2.0128851 | 0.027 |
| Phosphate Buffer | 411 | 48.912451 | 0.12  |

**Table S4** Individual components used to prepare lung simulant fluid (SLF) using the formula published by Gray et al.<sup>2</sup>

| Component                                           | MW  | mg/L | mM   | Manufacturer      | Lot        |
|-----------------------------------------------------|-----|------|------|-------------------|------------|
| NaCl                                                | 58  | 6.8  | 6.8  | Fisher Scientific | 126479     |
| NH <sub>4</sub> Cl                                  | 53  | 5.3  | 5.3  | Fisher Scientific | 158618     |
| NaHCO <sub>3</sub>                                  | 84  | 2.3  | 2.3  | Sigma Aldrich     | 088K014411 |
| H <sub>3</sub> PO <sub>4</sub>                      | 98  | 1.2  | 1.2  | Arcos Organics    | AO322150   |
| NaH <sub>2</sub> PO <sub>4</sub> • H <sub>2</sub> O | 138 | 1.7  | 1.7  | Fisher Scientific | 151189A    |
| Na <sub>2</sub> CO <sub>3</sub>                     | 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 <sub>2</sub> SO <sub>4</sub>                      | 98  | 0.51 | 0.51 | Fisher Scientific | 152112     |
| Na <sub>3</sub> -Citrate • 2H <sub>2</sub> O        | 258 | 0.59 | 0.59 | Sigma Aldrich     | 079K0044   |
| CaCl <sub>2</sub>                                   | 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.<sup>3</sup>

| Component                                                                                                 | MW            | mg/L | mM   | Manufacturer      | Lot      |
|-----------------------------------------------------------------------------------------------------------|---------------|------|------|-------------------|----------|
| Na <sub>2</sub> HPO <sub>4</sub>                                                                          | 142           | 142  | 1.0  | Sigma Aldrich     | 60790    |
| NaCl                                                                                                      | 58            | 6650 | 114  | Fisher            | 126479   |
| Na <sub>2</sub> SO <sub>4</sub>                                                                           | 142           | 71   | 0.50 | Fisher Scientific | 09505DE  |
| CaCl <sub>2</sub> • 2H <sub>2</sub> O                                                                     | 147           | 29   | 0.20 | Fisher Scientific | 096799   |
| Glycine                                                                                                   | 75            | 450  | 6.0  | Fisher Scientific | 1314121  |
| Potassium hydrogen phthalate (1-(HO <sub>2</sub> C)–2-(CO <sub>2</sub> K)–C <sub>6</sub> H <sub>4</sub> ) | 204           | 4085 | 20   | Arcos Organics    | AO237427 |
| Alkylbenzyltrimethylammonium chloride (ABCD)                                                              | N/A (polymer) | 50   | -    | Alpha Aesar       | X03A029  |



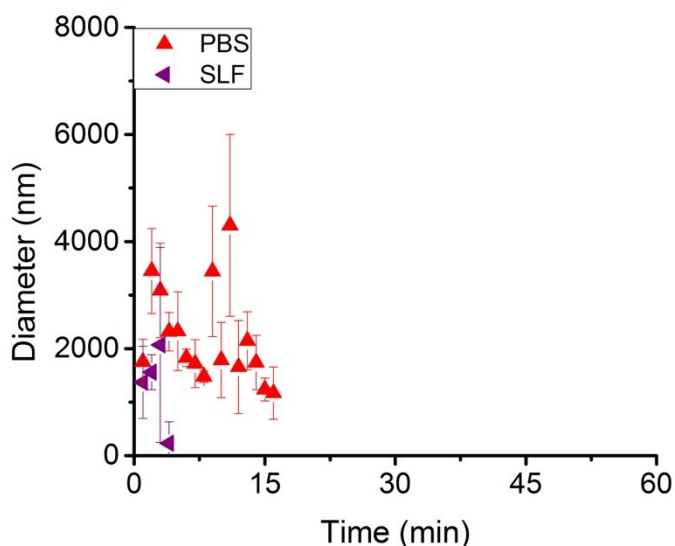
**Figure S1.** Additional morphology characterization of test and reference samples. (A) Crocidolite asbestos: as reported in Moalli et al. (1987)<sup>4</sup> and Sanchez et al. (2011)<sup>5</sup>. (B) Wollastonite: as reported in Bellman and Muhle (1994)<sup>6</sup> and Macdonald and Kane (1997).<sup>6,7</sup> (C) MoO<sub>3</sub> nanoribbon: SEM image obtained using a Zeiss LEO 1530 at 10 KeV. (D) MoO<sub>3</sub> 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 <sup>2</sup> /g) | Chemical Composition                                                                                                                   |
|------------------------------------------------------|-----------------------------|-------------------------|----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| MoO <sub>3</sub> nanoribbons                         | 10µm x 200nm x 10nm         | Small, lamellar strips  | 44.82                            | MoO <sub>3</sub>                                                                                                                       |
| MoO <sub>3</sub> nanoparticles                       | 80-5000 nm                  | Rectangular / irregular | 0.03-2                           | MoO <sub>3</sub>                                                                                                                       |
| Wollastonite (NYAD 1250 THOR) Belmann and Muhle 1994 | 0.4µm (1.42) x 1.7 (1.4) µm | Rod-like                | 3.94                             | CaSiO <sub>3</sub>                                                                                                                     |
| Crocidolite asbestos (Sanchez et al 2011)            | 2.8 (2.6) µm x 116 (112) nm | Rod-like                | 9.1                              | (Na <sub>2</sub> (Fe <sup>3+</sup> ) <sub>2</sub> (Fe <sup>2+</sup> ) <sub>3</sub> Si <sub>8</sub> O <sub>22</sub> (OH) <sub>2</sub> ) |

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

| MoO <sub>3</sub> concentration (µg/ml) | Mo Molarity (µM) | Na <sub>2</sub> MoO <sub>4</sub> concentration (µg/ml) |
|----------------------------------------|------------------|--------------------------------------------------------|
| 10                                     | 70               | 14.3                                                   |
| 50                                     | 350              | 71.5                                                   |
| 100                                    | 690              | 143                                                    |

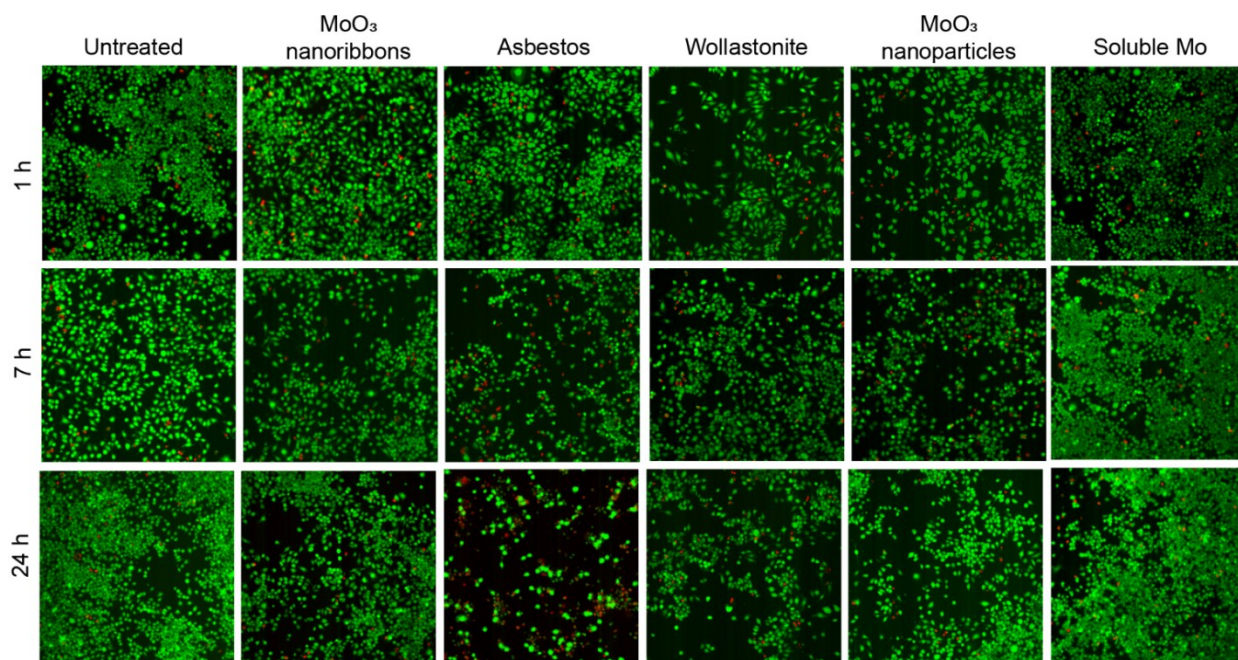


**Figure S2.** DLS stability test results for MoO<sub>3</sub> suspended in Phosphate Buffered Saline (PBS) and Simulated Lung Fluid (SLF) at a concentration of 500 mg/L. Both systems are buffered at pH 7.4 and contain no proteins (i.e. FBS). Three replicates were taken for each time point. Data shown reflects what was obtained prior to the instrument terminating data collection due to failing instrumental data quality criteria.

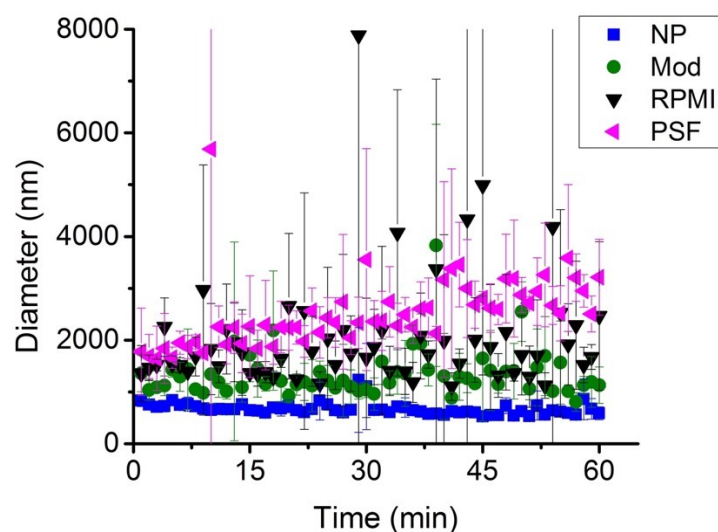
### MoO<sub>3</sub> Concentration Dependent Behavior

The observed dissolution and stability of MoO<sub>3</sub> in EPA moderately hard water can be explained by comparing total the variable total MoO<sub>3</sub> concentration to the fixed concentration of HCO<sub>3</sub><sup>-</sup> in Mod water. Bicarbonate is capable of buffering any H<sup>+</sup> produced through the dissolution of MoO<sub>3</sub>, which is expected based on the initial pH range of 7.4 to 7.8 for Mod water. The molar concentration of HCO<sub>3</sub><sup>-</sup> (added as NaHCO<sub>3</sub>) is fixed at 1.14 mol L<sup>-1</sup>. Complete dissolution of MoO<sub>3</sub> following the reaction stoichiometry of equation 1 would yield H<sup>+</sup> concentrations of 6.9

mol L<sup>-1</sup> for DLS experiments and 0.69 mol L<sup>-1</sup> for filtration experiments. Clearly, DLS experiments would produce enough H<sup>+</sup> to protonate all available HCO<sub>3</sub><sup>-</sup>, 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<sup>+</sup> produced through MoO<sub>3</sub> dissolution does not exceed the buffering capacity of bicarbonate and complete 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<sup>+</sup> (from MoO<sub>3</sub>) 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<sup>-1</sup> in NP, Mod, RPMI and PSF.

## References

- 1 EPA, Methods for Measuring the Acute Toxicity of Effluents and receiving Waters to Freshwater and Marine Organisms, 2002.
- 2 J. E. Gray, G. S. Plumlee, S. A. Morman, P. L. Higuera, J. G. Crock, H. A. Lowers and M. L. Witten, *Environ. Sci. Technol.*, 2010, **44**, 4782–4788.
- 3 A. B. Stefaniak, R. A. Guilmette, G. A. Day, M. D. Hoover, P. N. Breyse and R. C. Scripsick, *Toxicol. In Vitro*, 2005, **19**, 123–134.
- 4 P. A. Moalli, J. L. MacDonald, L. A. Goodglick and A. B. Kane, *Am. J. Pathol.*, 1987, **128**, 426–445.
- 5 V. C. Sanchez, P. Weston, A. Yan, R. H. Hurt and A. B. Kane, *Part. Fibre Toxicol.*, 2011, **8**, 17.
- 6 B. Bellmann and H. Muhle, *Environ. Health Perspect.*, 1994, **102**, 191–195.
- 7 J. L. Macdonald and A. B. Kane, *Toxicol. Sci.*, 1997, **38**, 173–183.