**Supporting information for the manuscript entitled**

**“Fibrillar vs Crystalline Nanocellulose Pulmonary Epithelial Cell Responses: Cytotoxicity or Inflammation?”**

Autumn L. Menas1#, Naveena Yanamala1#, Mariana T. Farcas1, Maria Russo3, Sherri Friend2, Philip M. Fournier4, Alexander Star4, Ivo Iavicoli5, Galina V. Shurin6, Ulla B. Vogel7, Bengt Fadeel8, Don Beezhold9, Elena R. Kisin1#, Anna A. Shvedova1,10**\***

1Exposure Assessment Branch/NIOSH/CDC, Morgantown, WV; 2Pathology & Physiology Research Branch/NIOSH/CDC, Morgantown, WV; 3Institute of Public Health, Section of Occupational Medicine, Catholic University of the Sacred Heart, Rome, Italy; 4Department of Chemistry, University of Pittsburgh, Pittsburgh, PA; 5Department of Public Health, Division of Occupational Medicine, University of Naples Federico II, Naples, Italy; 6Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA; 7National Research Centre for the Working Environment, Copenhagen, Denmark; 8Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 9Health Effects Laboratory Division/NIOSH/CDC, Morgantown, WV; 10Department of Physiology and Pharmacology, West Virginia University, Morgantown, WV

#) These authors have contributed equally

\*) Corresponding Author: Dr. Anna A. Shvedova, Exposure Assessment Branch (MS-2015), 1095 Willowdale Road, Morgantown, WV 26505 USA. Phone: (304) 285-6177. Fax: (304) 285-5938. E-mail: [ats1@cdc.gov](mailto:ats1@cdc.gov)

**Relevance of employed *in vitro* concentrations to realistic human exposures.**

Direct quantitative comparisons between in vitro and in vivo toxicological studies are difficult to make because of the enormous complexity of physiological, inflammatory and metabolic responses *in vivo* versus over-simplification of cell culture models lacking the whole body interactions. The effective dose of particles reaching the alveolar cells *in vivo* could be smaller than the deposited dose, partly due to the active clearance by macrophages and mucociliary transport, and partly due to the barrier provided by the mucus layer and the lung surfactant. However, it is important to assess, at least in semi-quantitative way, how may doses used in cell culture exposures in vitro relate or compare to realistic human exposures. Several mathematical models have been used by various groups to overcome challenges associated with translating the in vitro 1 and/or in vivo rodent studies to corresponding human equivalent exposures 2-4. Assuming that the particles investigated here will quickly settle onto the cells at the bottom of each well in the 96-well plate containing ~2 million cells, 100 μl of 300 μg/ml of particle would result in 0.0015 μg of deposited dose per cell. Using the total number of alveolar macrophages and Type-II epithelial cells of 5,990 ±1,990 and 32,900±13,600 million cells per lung for the humans 5, the equivalent human total lung macrophage and type-II epithelial cell burden would be ~9 and ~49 g, respectively. If an average diameter (DLS and TEM) = ~1 - 2 μm for various nanocellulose materials is used, minute ventilation of 20 L/minute and volume of 9.6 m3/day (20 L/min \* 0.001 m3/L \* 60 min/hr \* 8 hr/day) for a person working a 8 hr shift 6 and an alveolar deposition fraction of ~15%7-10, the equivalent alveolar macrophage and type-II cell burden in workers exposed at the OSHA Conc. of allowable exposure to respirable fraction of cellulose dust 5 mg/m3 would be achieved in approximately ~5 and ~26 years, respectively 2, 4. This indicates that the employed in vitro doses approximate feasible human occupational exposures and are well within the average working life time (<<45years). Notably, these estimates ignored several factors including the differences between the employed versus dose delivered to cells in vitro, equating accumulated doses over years with doses given all at once in the in vitro models.

**Given that:**

* **MSHA Conc. Of allowable exposure to cellulose based materials (A) =** 5 mg/m3 (respirable fraction)
* **Minute volume (B) =** 20 L/min = 20L/min \* 0.001 m3/L \* 60 min/hr \* 8hr/day = 9.6 m3/day
* **Average deposition efficiency of particles of size ~1-2mm (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 106) cells *(Stone et al., 1992)*

**Highest Concentration used *In vitro* :**

~2 x 106 A549cells were exposed to nanocellulose particles in a 96-well plates.

Total deposited dose per A549 or type-II cell = (45 μg/cm2 \* 0.32 cm2/well) / ((2 x 106)/ 96 wells)

= 0.0007 μg/cell

**Equivalent Exposure in humans:**

* **Deposited Concentration per Type-II cell =** ( Aerosol Conc. (A) X Exposure Duration X Minute Volume(B) X Alveolar Deposition Fraction (C) ) / Total number of Type-II Cells in the alveolar space (D)

(0.00073μg/cell)= (A x B x C x **duration**)/D

* **Exposure duration in humans =** (0.0007 μg/cellx D) / (A x B x C)

= (0.0007 μg/cellx (32,900 x 106 cells))/ (5000 μg/m3 x 9.6m3/day x 0.15)

= (23.03 x 106 μg) / (7200 μg/day)

= ~3,199 working days = ~640weeks = 12.3 years = **~12 years**

* **Similarly,**

Exposure duration in humans to achieve an equivalent lung burden of 0.00023 μg (i.e, at 15 μg/cm2) and 0.000023 μg (i.e, at 1.5 μg/cm2) per epithelial type II cell will be **~4 years** and **0.4 years**, respectively.

**Table S1.** Cytokine responses to nanocellulose exposure (24h, ng/mg protein)

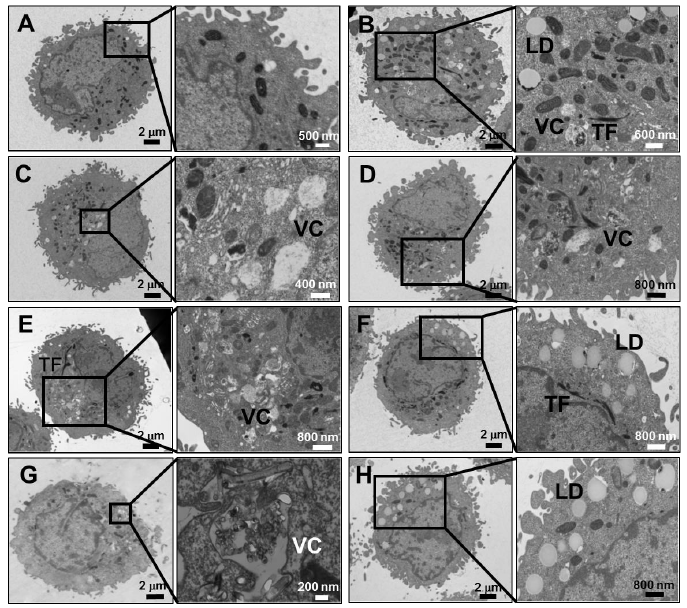
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Exposure** | **IL-1ra** | **IL-6** | **IL-8** | **IL-9** | **IL-10** | **IL-12(p70)** | **IL-17** | **Eotaxin** | **FGF basic** | **G-CSF** | **GM-CSF** | **IP-10** | **MCP-1** | **PDGF-bb** | **VEGF** |
| Control | ----------- | 0.108 ± 0.016 | 0.017 ± 0.002 | 0.504 ± 0.062 | 0.035 ± 0.005 | 0.080 ± 0.006 | 0.650 ± 0.052 | 0.360 ± 0.032 | 0.066 ± 0.004 | 0.223 ± 0.007 | 0.239 ± 0.017 | 0.343 ± 0.025 | 0.303 ± 0.065 | 0.859 ± 0.058 | 0.091 ± 0.006 | 6.147 ± 0.839 |
| Chitin | 1.5 µg/cm2 | 0.116 ± 0.030 | 0.021 ± 0.002 | 0.370 ± 0.022 | 0.0320 ± 0.0040 | 0.071 ± 14.56 | 0.542 ± 0.220 | 0.470 ± 0.056 | 0.058 ± 0.010 | 0.291 ± 0.053 | 0.207 ± 0.048 | 0.351 ± 0.022 | 0.451 ± 0.053 | 0.800 ± 0.246 | 0.095 ± 0.012 | 6.851 ± 2.830 |
| 15 µg/cm2 | 0.197 ± 0.018 \* | 0.013 ± 0.002 | 0.461 ± 0.061 | 0.067 ± 0.029 | 0.088 ± 0.017 | 0.464 ± 0.241 | 0.715 ± 0.236 | 0.078 ± 0.019 | 0.741 ± 0.049\* | 0.292 ± 0.033 | 0.487 ± 0.047 | 0.539 ± 0.097 | 0.660 ± 0.239 | 0.089 ± 0.011 | 5.089 ± 3.009 |
| 45 µg/cm2 | 0.102 ± 0.006 | 0.018 ± 0.001 | 0.796 ± 0.033 \* | 0.030 ± 0.001 | 0.111 ± 0.008\* | 1.085 ± 0.176\* | 0.403 ± 0.034 | 0.085 ± 0.009\* | 0.390 ± 0.069 | 0.323 ± 0.037 | 0.381 ± 0.006 | 0.501 ± 0.178 | 1.169 ± 0.006\* | 0.083 ± 0.018 | 16.115 ± 2.654\* |
| CNF | 1.5 µg/cm2 | 0.131 ± 0.013 | 0.021 ± 0.005 | 0.734 ± 0.023 | 0.045 ± 0.001 | 0.093 ± 0.003 | 0.850 ± 0.061 | 0.498 ± 0.061 | 0.074 ± 0.007 | 0.330 ± 0.023 | 0.331 ± 0.018 | 0.420 ± 0.003 | 0.623 ± 0.037\* | 0.710 ± 0.030 | 0.076 ± 0.013 | 9.736 ± 0.089\* |
| 15 µg/cm2 | 0.233 ± 0.032\* | 0.030 ± 0.006\* | 1.206 ± 0.098\* | 0.068 ± 0.009\* | 0.152 ± 0.004\* | 1.300 ± 0.072\* | 0.630 ± 0.061\* | 0.080 ± 0.017 | 1.624 ± 0.220\* | 0.701 ± 0.055\* | 0.608 ± 0.010\* | 0.941 ± 0.398 | 0.806 ± 0.021 | 0.236 ± 0.029\* | 11.126 ± 0.351\* |
| 45 µg/cm2 | 0.327 ± 0.016 \* | ND | 1.298 ± 0.055\* | 0.070 ± 0.030\* | 0.195 ± 0.033\* | 1.522 ± 0.128\* | 0.769 ± 0.329 | 0.117 ± 0.014\* | 1.983 ± 0.017\* | 0.933 ± 0.064\* | 0.890 ± 0.137\* | 0.636 ± 0.202 | 1.189 ± 0.031\* | 0.192 ± 0.084 | 14.527 ± 2.361\* |
| CNC Powder FD | 1.5 µg/cm2 | 0.195 ± 0.058 | 0.026 ± 0.002 | 0.910 ± 0.258 | 0.093 ± 0.025\* | 0.116 ± 0.064 | 0.876 ± 0.614 | 0.770 ± 0.035\* | 0.071 ± 0.009 | 0.709 ± 0.205 | 0.330 ± 0.026 | 0.595 ± 0.044\* | 0.577 ± 0.058\* | 1.607 ± 0.479 | 0.131 ± 0.013 | 10.541 ± 7.057 |
| 15 µg/cm2 | 0.211 ± 0.025\* | 0.072 ± 0.011\* | 2.020 ± 0.360\* | 0.067 ± 0.013\* | 0.080 ± 0.011 | 0.589 ± 0.202 | 0.347 ± 0.042 | 0.053 ± 0.011 | 0.418 ± 0.218 | 0.358 ± 0.026 | 0.498 ± 0.018\* | 0.582 ± 0.182 | 1.354 ± 0.137\* | 0.104 ± 0.023 | 9.644 ± 4.139 |
| 45 µg/cm2 | 0.145 ± 0.022 | 0.117 ± 0.013\* | 2.315 ± 0.269 \* | 0.062 ± 0.011\* | 0.061 ± 0.003 | 0.567 ± 0.031 | 0.498 ± 0.068\* | 0.070 ± 0.020 | 0.327 ± 0.204 | 0.485 ± 0.067\* | 0.470 ± 0.005\* | 0.404 ± 0.124 | 1.312 ± 0.144\* | 0.125 ± 0.009 | 5.653 ± 1.947 |
| CNC Powder SD | 1.5 µg/cm2 | 0.353 ± 0.066\* | 0.049 ± 0.002\* | 1.031 ± 0.021\* | 0.099 ± 0.019\* | 0.221 ± 0.028\* | 2.641 ± 0.110\* | 0.565 ± 0.107 | 0.147 ± 0.000\* | 1.186 ± 0.043\* | 0.675 ± 0.086\* | 1.080 ± 0.021\* | 0.856 ± 0.197\* | 2.853 ± 0.365 | 0.249 ± 0.011\* | 35.286 ± 7.393 |
| 15 µg/cm2 | 0.213 ± 0.034\* | 0.043 ± 0.008\* | 0.807 ± 0.142\* | 0.103 ± 0.029\* | 0.124 ± 0.027\* | 0.808 ± 0.279 | 0.348 ± 0.140 | 0.138 ± 0.033\* | 0.497 ± 0.171 | 0.617 ± 0.087\* | 0.927 ± 0.056\* | 1.025 ± 0.107\* | 1.559 ± 0.374 | 0.170 ± 0.051 | 10.099 ± 5.109 |
| 45 µg/cm2 | 0.262± 0.038\* | 0.049 ± 0.001\* | 0.725 ± 0.169\* | 0.130 ± 0.034\* | 0.118 ± 0.019 | 0.639 ± 0.271 | 0.956 ± 0.063\* | 0.142 ± 0.015\* | 0.892 ± 0.298\* | 0.514 ± 0.064\* | 1.025 ± 0.068\* | 1.010 ± 0.266\* | 1.005 ± 0.179 | 0.200 ± 0.060\* | 10.099 ± 5.484 |
| CNC Gel | 1.5 µg/cm2 | 0.244 ± 0.016\* | 0.022 ± 0.005 | 1.071 ± 0.006 \* | 0.092 ± 0.002\* | 0.211 ± 0.021\* | 2.667 ± 0.117\* | 1.179 ± 0.037\* | 0.056 ± 0.011 | 1.297 ± 0.112\* | 0.774 ± 0.099\* | 1.096 ± 0.011\* | 0.749 ± 0.237 | 3.966 ± 0.044\* | 0.162 ± 0.036\* | 35.382 ± 2.255\* |
| 15 µg/cm2 | 0.239 ± 0.066\* | 0.067 ± 0.007\* | 1.025 ± 0.121\* | 0.082 ± 0.005\* | 0.155 ± 0.011\* | 1.348 ± 0.042\* | 0.992 ± 0.029\* | 0.096 ± 0.015\* | 0.488 ± 0.032\* | 0.496 ± 0.022\* | 0.869 ± 0.042\* | 0.864 ± 0.133\* | 2.088 ± 0.068\* | 0.183 ± 0.024\* | 20.043 ± 1.605\* |
| 45 µg/cm2 | 0.186 ± 0.018\* | 0.053 ± 0.003\* | 1.190 ± 0.057\* | 0.083 ± 0.011\* | 0.127 ± 0.009\* | 1.039 ± 0.096\* | 1.040 ± 0.063\* | 0.125 ± 0.020\* | 0.342 ± 0.036\* | 0.550 ± 0.028\* | 0.916 ± 0.049\* | 0.842 ± 0.023\* | 1.410 ± 0.141\* | 0.206 ± 0.038\* | 14.473 ± 0.732\* |
| NCF Powder | 1.5 µg/cm2 | 0.044 ± 0.001 | 0.003 ± 0.001 | 0.588 ± 0.056 | 0.025 ± 0.004 | 0.050 ± 0.002 | 0.451 ± .0.075 | 0.163 ± 0.008 | ND | 0.394 ± 0.018\* | 0.164 ± 0.011 | 0.230 ± 0.006 | 0.222 ± 0.051 | 0.773 ± 0.009 | 0.051 ± 0.011 | 3.976 ± 0.054 |
| 15 µg/cm2 | 0.066 ± 0.014 | 0.006 ± 0.003 | 0.304 ± 0.056 | 0.044 ± 0.014 | 0.031 ± 0.006 | 0.319 ± 0.170 | 0.086 ± 0.031 | 0.046 ± 0.001 | 0.409 ± 0.023\* | 0.152 ± 0.017 | 0.298 ± 0.006 | 0.242 ± 0.045 | 0.456 ± 0.127 | 0.069 ± 0.016 | 1.676 ± 0.891 |
| 45 µg/cm2 | 0.058 ± 0.014 | 0.004 ± 0.001 | 0.409 ± 0.036 | 0.015 ± 0.002 | 0.026 ± 0.004 | 0.224 ± 0.020 | 0.134 ± 0.013 | 0.035 ± 0.003 | 0.126 ± 0.018 | 0.170 ± 0.007 | 0.229 ± 0.007 | 0.128 ± 0.020 | 0.544 ± 0.055 | 0.076 ± 0.010 | 2.172 ± 0.375 |
| NCF Gel | 1.5 µg/cm2 | 0.156 ± 0.014 | 0.004 ± 0.001 | 0.368 ± 0.007 | 0.052 ± 0.004\* | 0.033 ± 0.002 | 0.193 ± 0.024 | ND | 0.029 ± 0.002 | 1.264 ± 0.054\* | 0.204 ± 0.001 | 0.364 ± 0.037 | 0.277 ± 0.022 | 0.563 ± 0.001 | ND | 1.528 ± 0.271 |
| 15 µg/cm2 | 0.159 ± 0.043 | 0.014 ± 0.005 | 1.544 ± 0.219\* | 0.068 ± 0.004\* | 0.117 ± 0.001\* | 1.174 ± 0.071\* | 0.386 ± 0.046 | 0.089 ± 0.014 | 1.546 ± 0.054\* | 0.426 ± 0.027\* | 0.776 ± 0.032\* | 0.366 ± 0.028 | 1.732 ± 0.343\* | 0.227 ± 0.029\* | 8.071 ± 2.836 |
| 45 µg/cm2 | 0.073 ± 0.0.015 | 0.015 ± 0.003 | 0.796 ± 0.103 | 0.023 ± 0.003 | 0.034 ± 0.009 | 0.142 ± 0.012 | 0.318 ± 0.056 | 0.056 ± 0.003 | 0.322 ± 0.134 | 0.221 ± 0.054 | 0.383 ± 0.039 | 0.226 ± 0.007 | 0.996 ± 0.023 | 0.172 ± 0.032\* | 2.626 ± 0.894 |

27 different cytokines/chemokines were measured in the supernatants of A549 cells exposed to CNC and NCF for 24h. The values in the table represent an average of 4 replicates (ng/mg protein) ± SEM of two independent experiments in duplicates. The table contains cytokine levels at the three tested exposure doses (1.5, 15 and 45 μg/cm2). Significance shown as p<0.05 compared to control (\*). Only the cytokines that were able to be detected within the sensitivity range of the assay are included in the table. IL-1β, IL-2, IL-4, IL-5, IL-7, IL-13, IL-15, IFN, MIP-1α, MIP-1, RANTES, TNF-α were not detected (ND).

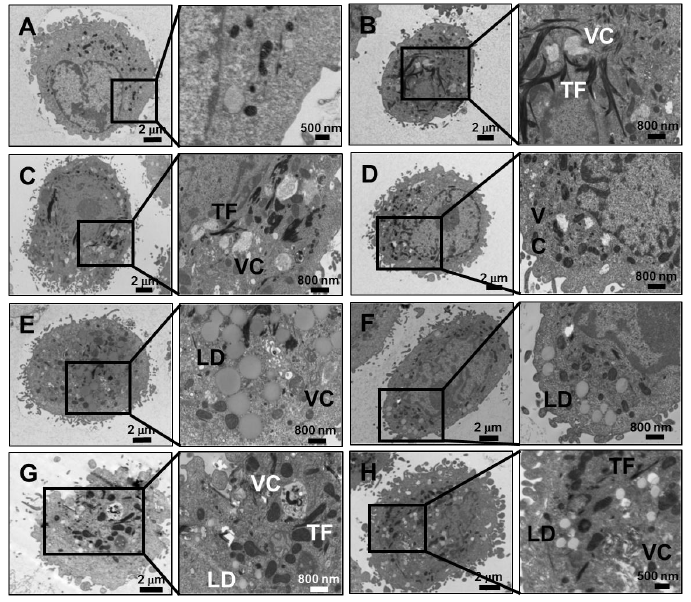
**Table S2.** Cytokine responses to nanocellulose exposure (72h, ng/mg protein)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Exposure** | **IL-1rα** | **IL-6** | **IL-7** | **IL-8** | **IL-10** | **IL-12(p70)** | **IL-13** | **FGF basic** | **G-CSF** | **GM-CSF** | **IFN-g** | **IP-10** | **MCP-1** | **PDGF-bb** | **VEGF** |
| Control | ------- | 0.169 ± 0.016 | 0.044 ± 0.004 | 0.166 ± 0.010 | 3.319 ± 0.195 | 0.899 ± 0.048 | 6.408 ± 0.097 | 0.225 ± 0.011 | 4.221 ± 1.374 | 0.301 ± 0.054 | 5.278 ± 0.195 | 0.717 ± 0.053 | 0.111 ± 0.012 | 6.234 ± 0.333 | 0.099 ± 0.015 | 61.210 ± 2.528 |
| Chitin | 1.5 µg/cm2 | 0.340 ± 0.082\* | 0.095 ± 0.003\* | 0.366 ± 0.016\* | 7.807 ± 1.171\* | 1.771 ± 0.112\* | 11.137 ± 0.089\* | 0.406 ± 0.011\* | 10.767 ± 0.5678 | 0.690 ± 0.109\* | 10.715 ± 0.363\* | 1.248 ± 0.001\* | 0.232 ± 0.019\* | 11.484 ± 0.560\* | 0.336 ± 0.033\* | 108.689 ± 3.844\* |
| 15 µg/cm2 | 0.236 ± 0.063 | 0.090 ± 0.005\* | 0.404 ± 0.057\* | 9.246 ± 0.660\* | 1.794 ± 0.101\* | 12.305 ± 0.198\* | 0.493 ± 0.031\* | 12.912 ± 1.036\* | 0.800 ± 0.086\* | 10.700 ± 0.044\* | 1.132 ± 0.160 | 0.260 ± 0.002\* | 10.528 ± 0.086\* | 0.207 ± 0.014\* | 112.479 ± 6.556\* |
| 45 µg/cm2 | 0.410 ± 0.086\* | 0.106 ± 0.013\* | 0.385 ± 0.000\* | 13.512 ± 0.226\* | 1.452 ± 0.106\* | 10.711 ± 0.532\* | 0.392 ± 0.043\* | 8.992 ± 0.503\* | 0.779 ± 0.083\* | 10.619 ± 1.256\* | 1.681 ± 0.087\* | 0.290 ± 0.113 | 11.339 ± 1.302\* | 0.231 ± 0.043\* | 89.682 ± 10.421\* |
| CNF | 1.5 µg/cm2 | 0.136 ± 0.035 | 0.022 ± 0.001 | 0.091 ± 0.004 | 1.092 ± 0.008 | 0.350 ± 0.030 | 2.327 ± 0.015\* | 0.093 ± 0.007 | 1.028 ± 0.004 | 0.133 ± 0.006 | 3.085 ± 0.068 | 0.401 ± 0.062 | 0.122 ± 0.026 | 1.738 ± 0.061\* | 0.051 ± 0.007 | 21.271 ± 0.088 |
| 15 µg/cm2 | 0.137 ± 0.010 | 0.031 ± 0.001 | 0.151 ± 0.010 | 3.339 ± 0.303 | 0.426 ± 0.049 | 3.069 ± 0.352\* | 0.131 ± 0.003 | 4.588 ± 0.264 | 0.273 ± 0.009 | 2.964 ± 0.310 | 0.437 ± 0.063 | 0.142 ± 0.001 | 1.758 ± 0.242\* | 0.125 ± 0.003 | 19.711 ± 0.825 |
| 45 µg/cm2 | 0.053 ± 0.014 | 0.022 ± 0.002 | 0.096 ± 0.001 | 1.924 ± 0.037 | 0.294 ± 0.030 | 2.146 ± 0.055\* | 0.087 ± 0.007 | 4.992 ± 0.171 | 0.219 ± 0.007 | 2.029 ± 0.008 | 0.311 ± 0.011 | 0.076 ± 0.017 | 0.915 ± 0.044\* | 0.075 ± 0.016 | 15.508 ± 1.805 |
| CNC powder FD | 1.5 µg/cm2 | 0.177 ± 0.122 | 0.186 ± 0.075\* | 0.496 ± 0.021\* | 31.631 ± 3.630\* | 3.551 ± 0.855\* | 21.359 ± 1.990\* | 0.532 ± 0.031\* | 24.628 ± 0.900\* | 0.810 ± 0.238\* | 28.404 ± 4.093\* | 1.392 ± 0.340\* | ND | 44.341 ± 3.299\* | 0.286 ± 0.080\* | 257.558 ± 38.575\* |
| 15 µg/cm2 | 1.082 ± 0.160\* | 0.771 ± 0.020\* | 0.771 ± 8.081\* | 93.999 ± 8.062\* | 2.619 ± 0.202\* | 17.204 ± 1.158\* | 0.712 ± 0.081\* | 11.783 ± 0.232\* | 2.261 ± 0.217\* | 37.256 ± 1.523\* | 2.226 ± 0.414\* | 0.847 ± 0.089\* | 100.786 ± 11.658\* | 0.712 ± 0.099\* | 191.741 ± 10.893\* |
| 45 µg/cm2 | 0.358 ± 0.112 | 0.847 ± 0.042\* | 0.370 ± 0.062\* | 125.367 ± 13.102\* | 1.483 ± 0.107\* | 9.991 ± 0.619\* | 0.395 ± 0.011\* | 2.408 ± 0.119 | 2.529 ± 0.076\* | 27.287 ± 1.145\* | 1.148 ± 0.362 | 0.318 ± 0.075\* | 61.251 ± 6.352\* | 0.297 ± 0.075\* | 115.568 ± 8.123\* |
| CNC powder SD | 1.5 µg/cm2 | 0.646 ± 0.169\* | 0.173 ± 0.021\* | 0.737 ± 0.097\* | 12.270 ± 0.930\* | 3.322 ± 0.306\* | 19.890 ± 1.318\* | 0.859 ± 0.019\* | 14.102 ± 0.797\* | 1.167 ± 0.057\* | 29.324 ± 1.958\* | 2.798 ± 0.172\* | 0.486 ± 0.200 | 22.394 ± 1.945\* | 0.482 ± 0.089\* | 243.744 ± 8.996\* |
| 15 µg/cm2 | 0.578 ± 0.112\* | 0.299 ± 0.027\* | 0.719 ± 0.031\* | 19.205 ± 1.801\* | 2.916 ± 0.215\* | 21.978 ± 1.533\* | 0.854 ± 0.060\* | 13.414 ± 0.289\* | 1.412 ± 0.080 | 38.093 ± 3.218\* | 2.391 ± 0.159\* | 0.329 ± 0.002\* | 27.465 ± 1.183\* | 0.411 ± 0.032\* | 248.737 ± 17.398\* |
| 45 µg/cm2 | 0.277 ± 0.079 | 0.287 ± 0.012\* | 0.385 ± 0.067\* | 17.830 ± 1.020\* | 1.778 ± 0.181\* | 12.126 ± 0.435\* | 0.478 ± 0.043\* | 3.055 ± 0.083 | 1.155 ± 0.266\* | 39.443 ± 1.002\* | 1.648 ± 0.411\* | 0.301 ± 0.003\* | 13.459 ± 0.743\* | 0.191 ± 0.023\* | 130.249 ± 11.280\* |
| CNC Gel | 1.5 µg/cm2 | 0.385 ± 0.006\* | 0.102 ± 0.028\* | 0.384 ± 0.093\* | 10.729 ± 1.666\* | 1.752 ± 0.549 | 10.111 ± 1.910 | 0.375 ± 0.069\* | 9.188 ± 0.857\* | 0.742 ± 0.179\* | 12.880 ± 5.213 | 1.207 ± 0.677 | 0.435 ± 0.005\* | 14.463 ± 2.019\* | 0.165 ± 0.084 | 123.427 ± 17.227\* |
| 15 µg/cm2 | 0.532 ± 0.098\* | 0.197 ± 0.007\* | 0.247 ± 0.009\* | 10.311 ± 0.093\* | 1.300 ± 0.041\* | 8.128 ± 0.455 | 0.342 ± 0.036\* | 2.853 ± 0.232 | 0.748 ± 0.040\* | 17.145 ± 0.411\* | 1.051 ± 0.082\* | 0.322 ± 0.080\* | 9.710 ± 0.869\* | 0.404 ± 0.027\* | 94.935 ± 6.371\* |
| 45 µg/cm2 | 0.467 ± 0.031\* | 0.257 ± 0.009\* | 0.230 ± 0.019\* | 9.354 ± 0.587\* | 1.081 ± 0.063\* | 6.694 ± 0.497 | 0.253 ± 0.010 | 0.859 ± 0.019 | 0.580 ± 0.054\* | 19.682 ± 0.656\* | 1.502 ± 0.198\* | 0.543 ± 0.120\* | 5.858 ± 0.186 | 0.291 ± 0.054\* | 68.481 ± 1.348 |
| NCF powder | 1.5 µg/cm2 | 0.028 ± 0.003 | ND | 0.076± 0.005 | 1.497 ± 0.202 | 0.313 ± 0.030 | 2.507 ± 0.309 | 0.082 ± 0.009 | 2.144 ± 0.028 | 0.154 ± 0.036 | 2.239 ± 0.355 | 0.226 ± 0.017 | 0.072 ± 0.001 | 2.384 ± 0.343 | 0.041 ± 0.020 | 18.805 ± 2.051 |
| 15 µg/cm2 | 0.019 ± 0.008 | ND | 0.061 ± 0.008 | 1.135 ± 0.042 | 0.209 ± 0.017 | 1.531 ± 0.084 | 0.063 ± 0.004 | 0.791 ± 0.005 | 0.105 ± 0.002 | 2.206 ± 0.157 | 0.162 ± 0.031 | 0.033 ± 0.012 | 2.107 ± 0.123 | 0.129 ± 0.013\* | 13.363 ± 0.494 |
| 45 µg/cm2 | 0.033 ± 0.015 | ND | 0.057 ± 0.003 | 1.330 ± 0.106 | 0.146 ± 0.017 | 1.123 ± 0.054 | 0.043 ± 0.004 | 35.35 ± 17.43 | 0.076 ± 0.008 | 2.347 ± 0.215 | 0.140 ± 0.029 | 0.020 ± 0.001 | 1.976 ± 0.025 | 0.527 ± 0.137\* | 10.122 ± 1.447 |
| NCF gel | 1.5 µg/cm2 | 0.023 ± 0.007 | ND | 0.064 ± 0.004 | 1.432 ± 0.326 | 0.264 ± 0.023 | 2.047 ± 0.229 | 0.096 ± 0.003 | 1.360 ± 0.042 | 0.111 ± 0.012 | 2.369 ± 0.439 | 0.181 ± 0.009 | 0.036 ± 0.010 | 2.743 ± 0.421 | 0.031 ± 0.013 | 17.295 ± 0.263 |
| 15 µg/cm2 | 0.030 ± 0.010 | 0.018 ± 0.003 | 0.073 ± 0.007 | 1.417 ± 0.040 | 0.226 ± 0.022 | 1.697 ± 0.056 | 0.075 ± 0.009 | 0.967 ± 0.088 | 0.086 ± 0.023 | 2.729 ± 0.409 | 0.175 ± 0.006 | 0.027 ± 0.002 | 3.03.19 ± 0.284 | 0.099 ± 0.020 | 15.997 ± 1.276 |
| 45 µg/cm2 | 0.043 ± 0.002 | 0.012 ± 0.002 | 0.040 ± 0.006 | 1.395 ± 0.163 | 0.127 ± 0.030 | 0.835 ± 0.240 | 0.037 ± 0.008 | 0.460 ± 0.048 | 0.065 ± 0.016 | 1.939 ± 0.144 | 0.149 ± 0.023 | 0.017 ± 0.002 | 1.986 ± 0.499 | 0.173 ± 0.029\* | 7.681 ± 2.783 |

27 different cytokines/chemokines were measured in the supernatants of A549 cells exposed to CNC and NCF for 72h. The values in the table represent an average of 4 replicates (ng/mg protein) ± SEM of two independent experiments in duplicates. The table contains cytokine levels at the three tested exposure doses (1.5, 15 and 45 μg/cm2). Significance shown as p<0.05 compared to control (\*). Only the cytokines that were able to be detected within the sensitivity range of the assay are included in the table. IL-1β, IL-2, IL-4, IL-5, IL-9, IL-15, IL-17, MIP-1, MIP-1, Eotaxin, RANTES, TNF-α were not detected (ND).



**Figure S1.** Morphological changes induced by exposure to nanocellulose for 24 h. Representative TEM images of A549 cells exposed to 45 μg/cm2 of each nanoparticle for 24h where A - control cells, B – cells exposed to CNC powder FD, C – cells exposed to CNC powder SD, D - cells exposed to CNC gel, E - cells exposed to NCF powder, F - cells exposed to NCF gel, G - cells exposed to carbon nanofibers, and H - cells exposed to chitin. Exposed cells show an increased number of vacuoles (VC), tonofilaments (TF), and lipid droplets (LD).



**Figure S2.** Morphological changes induced by exposure to nanocellulose for 72h. Representative TEM images of A549 cells exposed to 45 μg/cm2 of each nanoparticle for 72h where A - control cells, B – cells exposed to CNC powder FD, C – cells exposed to CNC powder SD, D - cells exposed to CNC gel, E - cells exposed to NCF powder, F - cells exposed to NCF gel, G - cells exposed to carbon nanofibers, and H - cells exposed to chitin. Exposed cells show an increased number of vacuoles (VC), tonofilaments (TF), and lipid droplets (LD).



**Figure S3: Hierarchical Clustering Analysis of cytokine profiles in A549 cells exposed to various crystalline nanocellulose (CNC) and nanocellulose fiber (NCF) materials based on Ward.D2 linkage method.** The samples of A549 cells exposed to different concentrations of CNC and NCF along with respective controls (chitin and CNF), along with missing values, were clustered based on the Euclidean distance metric and ward.D2 clustering method at (A) 24h and (B) 72h post exposure time points. The heat map colors represent log2 transformed fold change values of cytokines relative to the minimum and maximum of all values, increasing from red to green, in each case. A key showing the range of values at each post exposure time point is also shown in the figure. White color represents missing-values or cytokines whose levels were below detections limits.

**References:**

1. Gangwal, S.; Brown, J. S.; Wang, A.; Houck, K. A.; Dix, D. J.; Kavlock, R. J.; Hubal, E. A., Informing selection of nanomaterial concentrations for ToxCast in vitro testing based on occupational exposure potential. *Environmental health perspectives* **2011,** 119, (11), 1539-46.

2. Erdely, A.; Dahm, M.; Chen, B. T.; Zeidler-Erdely, P. C.; Fernback, J. E.; Birch, M. E.; Evans, D. E.; Kashon, M. L.; Deddens, J. A.; Hulderman, T.; Bilgesu, S. A.; Battelli, L.; Schwegler-Berry, D.; Leonard, H. D.; McKinney, W.; Frazer, D. G.; Antonini, J. M.; Porter, D. W.; Castranova, V.; Schubauer-Berigan, M. K., Carbon nanotube dosimetry: from workplace exposure assessment to inhalation toxicology. *Part Fibre Toxicol* **2013,** 10, (1), 53.

3. Sargent, L. M.; Porter, D. W.; Staska, L. M.; Hubbs, A. F.; Lowry, D. T.; Battelli, L.; Siegrist, K. J.; Kashon, M. L.; Mercer, R. R.; Bauer, A. K.; Chen, B. T.; Salisbury, J. L.; Frazer, D.; McKinney, W.; Andrew, M.; Tsuruoka, S.; Endo, M.; Fluharty, K. L.; Castranova, V.; Reynolds, S. H., Promotion of lung adenocarcinoma following inhalation exposure to multi-walled carbon nanotubes. *Part Fibre Toxicol* **2014,** 11, 3.

4. NIOSH[2010] *Current Intelligence Bulletin: Occupational Exposure to Carbon Nanotubes and Nanofibers*; U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health,: Cincinnati, OH, 2010; pp DHHS (NIOSH) Docket Number: NIOSH 161-A; Available at <http://www.cdc.gov/niosh/docket/review/docket161A/>.

5. Stone, K. C.; Mercer, R. R.; Gehr, P.; Stockstill, B.; Crapo, J. D., Allometric relationships of cell numbers and size in the mammalian lung. *Am J Respir Cell Mol Biol* **1992,** 6, (2), 235-43.

6. Galer, D. M.; Leung, H. W.; Sussman, R. G.; Trzos, R. J., Scientific and practical considerations for the development of occupational exposure limits (OELs) for chemical substances. *Regul Toxicol Pharmacol* **1992,** 15, (3), 291-306.

7. *Multiple Path Particle Dosimetry Model (MPPD v 1.0): A Model for Human and Rat Airway Particle Dosimetry.*; National Institute for Public Health and the Environment (RIVM): Bilthoven, The Netherlands. , 2002.

8. Anjilvel, S.; Asgharian, B., A multiple-path model of particle deposition in the rat lung. *Fundam Appl Toxicol* **1995,** 28, (1), 41-50.

9. Bates, D. V.; Fish, B. R.; Hatch, T. F.; Mercer, T. T.; Morrow, P. E., Deposition and retention models for internal dosimetry of the human respiratory tract. Task group on lung dynamics. *Health physics* **1966,** 12, (2), 173-207.

10. ICRP, Human Respiratory Tract Model for Radiological Protection. In Ann. ICRP 1994; Vol. 24