Supplemental Material for:

**Acrolein inhalation alters myocardial synchrony and performance at and below exposure concentrations that cause ventilatory responses**

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**Table SI. Bronchoalveolar Lavage Fluid Cell Differentials**

|  |  |  |  |
| --- | --- | --- | --- |
|  | *Cells/mL x 104 (mean ± SD, n=6)* | | |
|  | **Filtered Air (FA)** | **0.3 ppm Acrolein** | **3.0 ppm Acrolein** |
| Total Cells | 7.07 ± 2.18 | 4.59 ± 1.24 | 7.47 ± 2.76 |
| Macrophages | 6.81 ± 2.09 | 4.46 ± 1.21 | 6.94 ± 2.36 |
|  | *Cells/mL x 102 (mean ± SD, n=6)* | | |
| Neutrophils | 5.83 ± 4.62 | 5.17 ± 3.76 | 4.83 ± 5.46 |
| Eosinophils | 3.50 ± 3.89 | 1.67 ± 2.66 | 4.17 ± 7.44 |
| Lymphocytes | 17.0 ± 9.30 | 5.83 ± 1.60 | 43.8 ± 55.24\*† |
| \*for *p<0.05* vs. FA; †for *p<0.05* vs. 0.3 ppm Acrolein; BAL collected 24 hours after exposure | | | |

**Table SII. Bronchoalveolar Lavage Fluid Analyses**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | **Filtered Air (FA)** | **0.3 ppm Acrolein** | **3.0 ppm Acrolein** |
| **Total Protein** | *µg/mL* | 63.9 ± 13.7 | 57.0 ± 5.7 | 80.8 ± 38.9 |
| **Albumin** | *µg/mL* | 7.2 ± 2.6 | 6.6 ± 1.2 | 7.6 ± 2.1 |
| **LDH** | *U/L* | 13.5 ± 4.2 | 23.1 ± 7.3 | 34.6 ± 33.7 |
| **NAG** | *U/L* | 3.6 ± 1.0 | 3.4 ± 0.8 | 4.5 ± 1.6 |
| **Total SOD** | *U/mL* | 1.1 ± 0.8 | 0.7 ± 0.2 | 2.3 ± 2.1 |
| **Mn SOD** | *U/mL* | 0.6 ± 0.5 | 0.4 ± 0.1 | 1.4 ± 1.3 |
| **CuZn SOD** | *U/mL* | 0.5 ± 0.3 | 0.3 ± 0.1 | 0.9 ± 0.8 |
| **TAS** | *µM* | 47 ± 40 | 25 ± 61 | 60 ± 49 |
| **GPX** | *IU/µL* | 130 ± 10 | 121 ± 7 | 158 ± 33† |
| **GTR** | *IU/µL* | 7.2 ± 2.7 | 7.8 ± 2.1 | 13 ± 3.8 |
| †for p<0.05 vs. 0.3 ppm Acrolein; BAL collected 24 hours after exposure; data are mean ± SD | | | | |
| MIA – micro-albumin; LDH – lactate dehydrogenase; NAG – N-acetyl-β-D-glucosaminidase; SOD – super oxide dismutase; TAS – total antioxidant status; GPX – glutathione peroxidase; GTR – glutathione reductase | | | | |



**Fig. S1: Ventilation data during complete baseline and exposure periods.** Baseline period occurred from the -5 minutes to the 0 minute, the dashed line at zero denotes the onset of exposure, and then the exposure lasted from 0 – 180 minutes. (A) Breathing frequency; (B) Inspiratory time; (C) Tidal volume; (D) Expiratory time; (E) Minute volume; (F) Expiratory time/inspiratory time ratio. Data are mean ± SD.



**Fig. S2: Exposure to acrolein altered relationship between end systolic volume (ESV) and end diastolic volumes (EDV).** Each data point represents the ESV and EDV value calculated from the analysis of 3 consecutive beats. We analyzed 6 groups of 3 beats were for each mouse (n = 6) per exposure group, totaling 36 data points per panel. Across time in the FA group (A, B, and C) and before exposure (Exposure – 24 hr) to acrolein at 0.3 ppm and 3.0 ppm (D and G), the slope and R2 were relatively similar, and ESV and EDV were correlated. After exposure to acrolein at 0.3 ppm (E and F) slope and R2 decreased, but ESV and EDV remained correlated. With 3.0 ppm acrolein at Exposure + 1 hr (H) slope and R2 decreased, but ESV and EDV were still correlated. With 3.0 ppm acrolein at Exposure + 24 hr slope and R2 decreased dramatically, and ESV and EDV were not correlated. Data plots were fit with a linear regression and tested for correlation and change in slope across concentration and time-point. Correlation p-values are provided. Significant changes in slope were marked with \*for p < 0.05 vs. FA; ‡for p < 0.05 vs. Exp – 24 hr.