

# **Pulmonary Toxicity Assessment after a Single Intratracheal Inhalation of Chlorhexidine Aerosol in Mice**

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## **Summary of Supporting Information**

### **Materials and methods:**

#### **ELISA**

According to the protocol of the ELISA kit, the levels of Hydroxyproline in BALF of the control group and CHG exposure group were detected. Hydroxyproline (CSB-E08839m) ELISA kits were purchased from CUSABIO (Wuhan, <https://www.cusabio.com/>, China). Briefly, add 100  $\mu$ l of standard and sample to be tested into the wells respectively, mix well, put on the plate sticker, incubate at 37 °C for two hours, discard the liquid in the wells, add 100  $\mu$ l of biotin labelling antibody working solution, put on the plate sticker, incubate at 37 °C for one hour, discard the liquid in the wells. Wash three times with 200  $\mu$ l washing solution, 2 minutes each, and shake dry. Add 100  $\mu$ l of horseradish peroxidase labelled affinity protein working solution to each well, affix the plate sticker and incubate at 37 °C for one hour. Discard the liquid in the wells, wash with 200  $\mu$ l of washing solution five times, two each time and shake dry. Add 90  $\mu$ l of the substrate solution, develop the color at 37 °C for 15-30min, add 50  $\mu$ l of termination solution, measure the optical density (OD) at 450 nm after 5 min, and calculate the concentration of each well. Samples from three animals per group were measured to derive the Mean  $\pm$  SD.

## Supplemental Tables and Figures:

Table S1. Primer sequences for mouse samples used in qPCR (5' to 3').

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
<i>Muc5ac</i>	TCTACTGACTGCACCAACACA	TCCGTCAGTCCACACTTTCG
<i>Muc5b</i>	CTGAAGACCTGTCGGAACCCAA	GCCACACACTTCATCTGGTCCT
<i>P4ha3</i>	TGCAAGTGGAGTACCGCATCAG	CACCTGGAGATACTCTGCGTAG
<i>Bpifa1</i>	GGTCTTGTGCAGAGTCCTGATG	CTTTCACGGCTAAGACTTCTGCA
<i>Bpifb1</i>	CAGGCACTGAAGGACCATGATG	GGATGTTAGCAGAGGTGACCTTC
<i>β-actin</i>	CATTGCTGACAGGATGCAGAAGG	TGCTGGAAGGTGGACAGTGAGG

Table S2. Mortality of chlorhexidine-exposed mice at different concentrations

Dose (%)	Death	Alive	Total	Mortality (%)
0	0	13	13	0
0.125	0	12	12	0
0.25	9	19	28	32.1
0.5	12	16	28	42.9
1	7	3	10	70.0

Table S3. Percentage of inflammatory cells in bronchoalveolar lavage fluid

Group	Macrophages	Lymphocytes	Neutrophils
Control	89.72±1.12	9.51±1.21	0.78±0.31
0.125%	88.35±1.14	10.68±0.65	0.97±0.82
0.25%	85.21±1.42*	13.11±1.21*	1.68±0.53
0.5%	85.61±1.58*	13.54±1.67*	0.85±0.12

\* $P < 0.05$ , compared with control group.

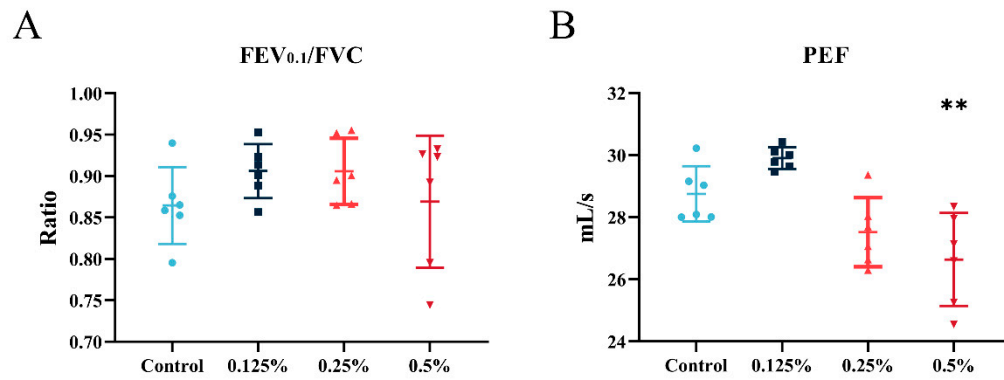


Figure S1. Chlorhexidine decreased pulmonary function in mice. (A) FVE<sub>0.1</sub>/FVC and (B) Peak expiratory flows (PEF) were measured using a NPFE model via the flexiVent FX system. Data were analyzed by one-way ANOVA with Dunnett's post-hoc test (\*\*  $P < 0.01$ ).

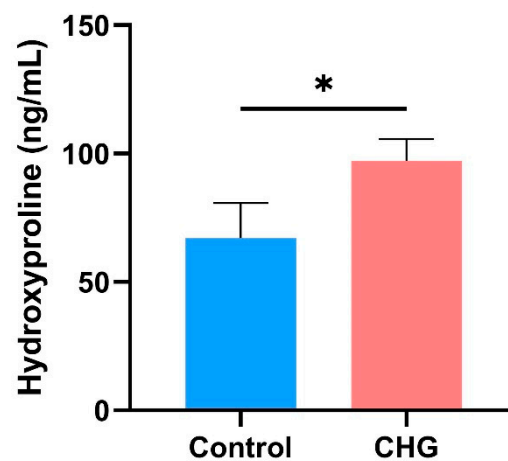


Figure S2. ELISA detection of hydroxyproline in BALF of control and CHG exposure groups. Data are presented as mean  $\pm$  SD ( $n = 3$ , \*  $P < 0.05$ ).